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PROFESSOR J. W. W. STEPHENS, M.D.Cantab., F.R.S.

PROFESSOR R. NEWSTEAD, M.Sc., J.P., F.R.S., A.L.S., F.E.S., Hon. F.R.H.S.

PROFESSOR WARRINGTON YORKE, M.D.

PROFESSOR B. BLACKLOCK, M.D.

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CHARLES NATHANIEL ROTHSCHILD, younger son of the first Lord Rothschild, was born in 1877 and died on October 12th, 1923, at the age of 46. He was educated at Harrow and Cambridge where he took Part I of the Natural Science Tripos. Although by profession an active member of the firm, N. M. Rothschild & Sons, he became one of the leading British Entomologists and devoted himself more particularly to the study of Siphonaptera. Before his time, entomologists, with the exception of Taschenberg and C. F. Baker, had neglected the study of this group.

Rothschild's researches on Ectoparasites have been of very great value to those concerned with the problems of the transmission of plague by fleas.

His collection, containing as it does a large number of types, is unrivalled, and has been bequeathed to the Trustees of the British Museum (Natural History), where it is available for study.

We, ourselves, wish to place on record our warm appreciation of the services rendered to the Liverpool School of Tropical Medicine in the determination of material and in the contribution of scientific papers

A MALARIA PARASITE OF THE CHIMPANZEE

BY

B. BLACKLOCK

AND

S. ADLER

From the Sir A. L. Jones Research Laboratory, Freetown

(*Received for publication* 10 *December*, 1923)

Reichenow (1920) described in chimpanzees in the Cameroons malaria parasites, indistinguishable from the three species which occur in human beings, and suggested that the chimpanzee is naturally affected with human malaria. Mesnil and Roubaud's successful inoculation of *Plasmodium vivax* into a chimpanzee supports Reichenow's conclusion as far as *P. vivax* is concerned.

Mesnil and Roubaud failed to infect a chimpanzee with *Plasmodium falciparum*, either by direct inoculation, or by infective mosquitoes. This experiment, however, cannot be taken as conclusive, as Reichenow and Adler have produced evidence to show that chimpanzees acquire immunity to malaria, after attacks in early life. A conclusive experiment could only be carried out on a very young animal which had not yet been attacked with malaria.

We were able to carry out such an experiment on October 30, 1922. A young male chimpanzee three months old was under observation since September 11, 1922, and its blood on repeated examination was negative; 3 c.c. of human blood heavily infected with *Plasmodium falciparum* were citrated and injected subcutaneously into the animal. The injection was given two and one-half hours after the blood was withdrawn from the human case.

The animal did not become infected with *P. falciparum*, and this, in conjunction with Blacklock and Adler's (1922) failure to infect two Europeans with infected chimpanzee's blood is evidence

that *Plasmodium falciparum* is a different species from the similar parasite which occurs in chimpanzee's blood. We, therefore, propose the name of *Plasmodium reichenowi* for the latter, in consideration of the fact that this observer first found the parasite in chimpanzees.

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ON THE ANATOMY OF *TAENIA FESTIVA*, RUDOLPHI, 1819

BY

G. THEILER.

From the Parasitology Department of the Liverpool School of Tropical Medicine

(*Received for publication* 11 *December*, 1923)

CITTOTAENIA FESTIVA (Rudolphi, 1819).

Synonym:—*Moniezia festiva*, Blanchard, 1891.

In 1819 Rudolphi described a cestode, *Taenia festiva*, from the bile duct and gall bladder of *Halmaturi gigantei* (= *Macropus giganteus*). This was later figured by Bremser in 1824, and was identified by Cobbold (1879) as occurring in *Macropus derbyanus*. Blanchard, in 1891, included it in his genus *Moniezia*. As Rudolphi's description does not give sufficient anatomical details, it has remained in Cestode Classification as a doubtful *Moniezia*.

Since 1819 other cestodes with bilateral pores have been described from kangaroos and allied animals, viz.:—

Taenia fimbriata, Krefft, 1873 = *Taenia? krefftii*, Johnston, 1909.

Taenia bipapillosa, Leidy, 1875, from a wombat.

Cittotaenia zschokkei, Janicki, 1905, from *Macropus* sp.?

Moniezia diaphana, Zschokke, 1907, from **Phaseolomys wombat*.

Cittotaenia bancrofti, Johnston, 1912, from *Onychogale frenata*.

Cittotaenia lagorchestis, Lewis, 1914, from *Lagorchestes conspicillatus*.

Cittotaenia villosa, Lewis, 1914, from *Lagorchestes conspicillatus*.†

* Johnston (1911) questions the correct identification of this host or the locality from which the host is recorded, as *P. wombat* does not occur on the mainland but in Tasmania or the neighbouring island of the Bass Straits.

† *T. phalangistae*, Krefft (1873), from the common opossum need not any longer be taken into account, as it is not recognisable from the description.

Johnston (1909) stated that he had recently examined superficially some material from *Macropus derbyanus* which apparently consisted of *Moniezia festiva*. He also mentioned that he has in his possession a number of tapeworms agreeing externally with *Taenia bipapillosa*, taken from a wombat, *Phaseolomys mitchelli*, and remarked ' I hope that before long I shall be able to make known the structure of these two species of *Moniezia*.' In 1912 he described a *Cittotaenia*, *C. bancrofti*, from a wallaby, *Onychogale frenata*, but so far, neither in this nor any other publication, has he made further mention of his specimen of *M. bipapillosa*.

All the double-pored cestodes from *Monotremes* and Marsupials which have been carefully examined have proved to belong to some species of the genus *Cittotaenia*, e.g., *C. zschokkei*, *C. bancrofti*, *C. diaphana*, *C. lagorchestis*.

According to the data given of *T. fimbriata*, Krefft (= *T.? krefftii*, Johnston), we gather that we are dealing either with a *Moniezia* or a *Cittotaenia*, but further identification is impossible.

Leidy's description of *T. bipapillosa* is imperfect, but Johnston points out its similarity with the figures of *Taenia festiva* as given by Bremser. We may, therefore, either assume that *T. bipapillosa* is synonymous with *T. festiva* or, owing to lack of anatomical data, leave it altogether out of account.

Examination of Rudolphi's original description and of Bremser's drawings, reveals the fact that we may have ' an appearance as of four ovaries (uteri),' and that the ovaries (uteri) do not meet in the median field. At no stage in the development of a *Moniezia* is this appearance as of four uteri to be seen, nor do the uteri ever have a clear space in the median line. In a few species (e.g., *C. variabilis* and *C. diaphana*) of the genus *Cittotaenia*, however, the two uteri arise separately and may not meet in the median field. Under the circumstances we can assume that Rudolphi's *Taenia festiva* is a *Cittotaenia* and not a *Moniezia*.

This assumption is borne out by the study of cestode material collected by Dr. Bruck from a kangaroo in Golden Gate Park, California. Mr. Southwell, of the Liverpool School of Tropical Medicine, to whom it had been sent for identification, most kindly handed it over to me. The material, consisting of one complete worm and several fragments, presented upon external and superficial

examination all the characteristics of *Taenia festiva*, as described by Rudolphi, but upon detailed investigation proved to be a typical *Cittotaenia*, with the following characters:—

CITTOTAENIA FESTIVA (Rudolphi, 1819)

The worm is about 13 cm. long ('8 to 10 pollices,' Rudolphi) and up to 5 mm. broad ('2 to 3 lineas,' Rudolphi); fairly thick in the contracted portions, thin in some of the relaxed fragments ('thin and transparent,' Rudolphi). Pores bilateral, opening in the posterior portion of the lateral margins.

The segments vary in shape according to the state of contraction of the worm, and are broader than long. Mature segments average 2·6 mm. broad by 0·35 mm. long; gravid segments are 5 mm. broad by 0·72 mm. long. In the mature portions of the worm, the posterior border of each segment overlaps at least one-half of the next segment.

Head (fig. 1). This is a short truncate cone, 0·54 mm. in length, with its greatest diameter 0·68 mm. near the anterior end. A marked

FIG. 1. *Cittotaenia festiva*. Head.

lobing is present, so that each sucker is prominent (as figured by Bremser). A certain amount of contraction is apparent. The openings of the suckers are circular and face anteriorly. Viewed *en face*, the outline of the head is square, four lobed.

Neck. A deep constriction separates the head from the rest of the strobila. It is difficult to say whether a neck is present or not, as this portion of the specimen is very contracted.

Nervous system. Ventral to the genital canals.

Muscular system. Not very strongly developed; the longitudinal muscles do not show any arrangement in definite rows.

Excretory system (fig. 3). Ventral to the genital canals. The dorsal vessel lies dorso-lateral to the ventral vessel. In some segments there appear to be transverse canals to the dorsal vessels.

Male genitalia (fig. 2). *Testes.* The testes are arranged in two groups. They number on an average forty-eight to fifty-five on the left side and sixty-four to sixty-eight on the right side. They do

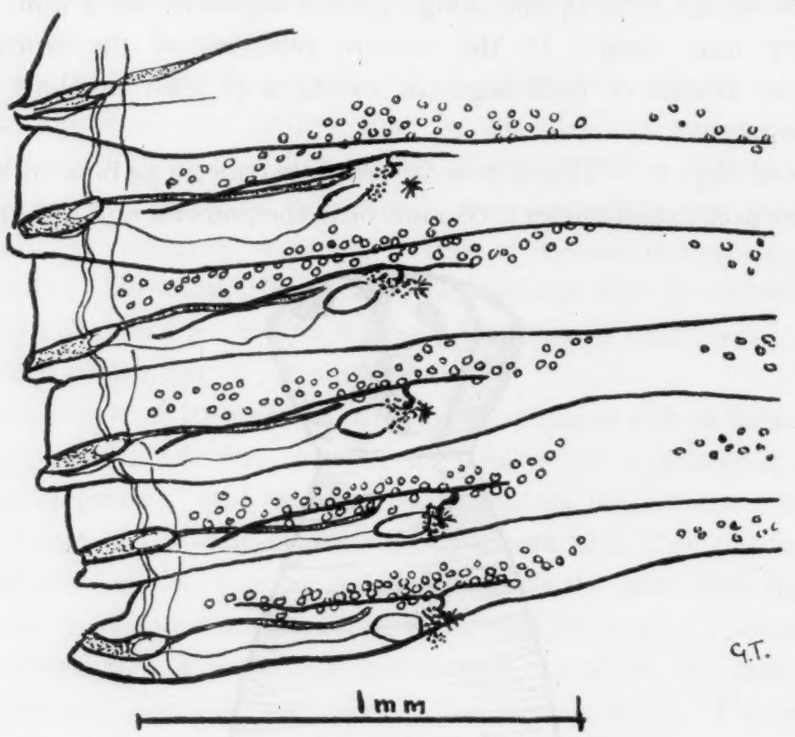

FIG. 2. *C. festiva. In toto* preparation of mature segment.

not meet in the median field, while laterally they reach to the longitudinal vessels. They are anterior and dorsal to the female genitalia.

Vas deferens (fig. 2). In the young segments the vas deferens appears as a straight narrow tube. Later, however, it enlarges into an elongate swelling before its entrance into the cirrus pouch; within the cirrus pouch it forms a seminal vesicle. Just median of the

enlargement, it describes a few coils and then continues as a straight and narrow tube (fig. 4). The cirrus pouch is large, slightly elongate and, before the seminal vesicle is filled with sperms, measures 240μ to 280μ by 44μ to 52μ. It reaches beyond the longitudinal canals, which it crosses dorsally.

Female genitalia (fig. 3). The fairly large mass of the shell gland adjoins the receptaculum seminis; median to this is the ovary and posteriorly the vitelline glands. The ovary may underlie the shell gland ventrally, the main bulk of the ovary lies ventral, and the vitelline gland is dorsal in position. The shell gland lies at a level between these two.

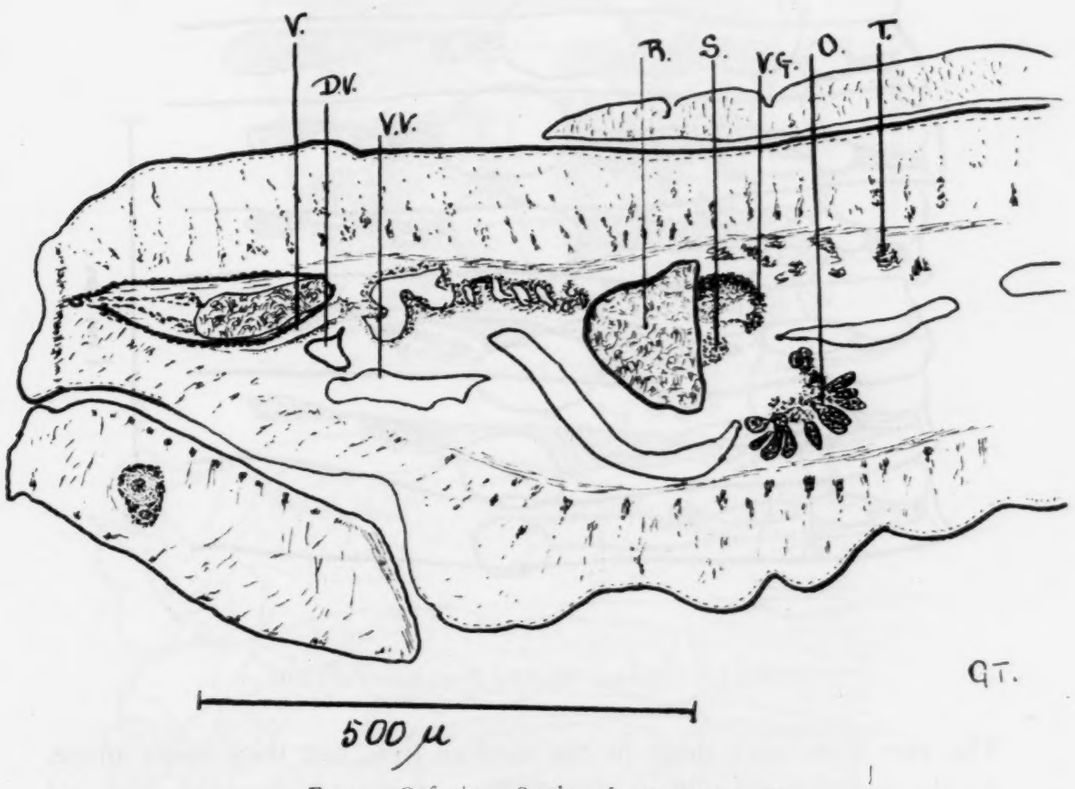

FIG. 3. *C. festiva.* Section of mature segment.

V.—Vagina; *D.V.*—Dorsal longitudinal vessel; *V.V.*—Ventral longitudinal vessel; *R.*—Receptaculum seminis; *S.*—Shell gland; *V.G.*—Vitellogene gland; *O.*—Ovary; *T.*—Testes.

Receptaculum seminis and vagina. The vagina is ventral and posterior to the cirrus pouch on both sides. It opens on the edge of the slight collar surrounding the male aperture. In older segments the vagina disappears. The receptaculum seminis may attain enormous proportions; when filled with sperms it extends from the anterior to the posterior margin of the segment (fig. 4).

In older proglottides the two receptacula are pushed posteriorly until they divide the posterior margin of the segment into three equal parts (fig. 5).

Oviduct. After receiving the vitelline duct the large oviduct runs anteriorly for a short distance, then dips and runs diagonally, ventrally and anteriorly, meeting the uterus at a right angle.

Uterus (figs. 2 and 4). This is at first a narrow tube (one to each set of genitalia), which eventually extends from the median line to the longitudinal vessels. It may cross these latter dorsally.

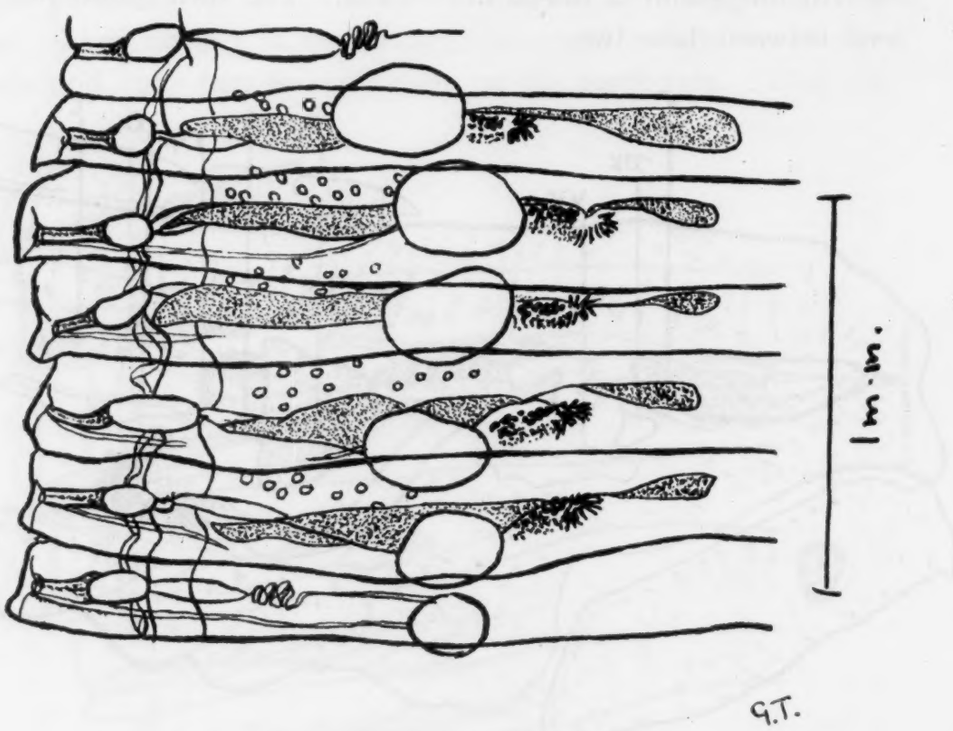

FIG. 4. *C. festiva.* Showing development of uterus.

The two uteri may meet in the median line, but they never unite. As the eggs develop the two branches of the uterus merely expand into two large pouches, giving in some segments the appearance of four separate units (as described by Rudolphi) until eventually they practically fill the whole segment (figs. 5 and 6).

Eggs. The eggs were not quite mature. Those examined in carbolic acid measured 46μ to 50μ in diameter, the bulb of the pyriform body 20μ to 23μ. The horns are long, coiled and tapering.

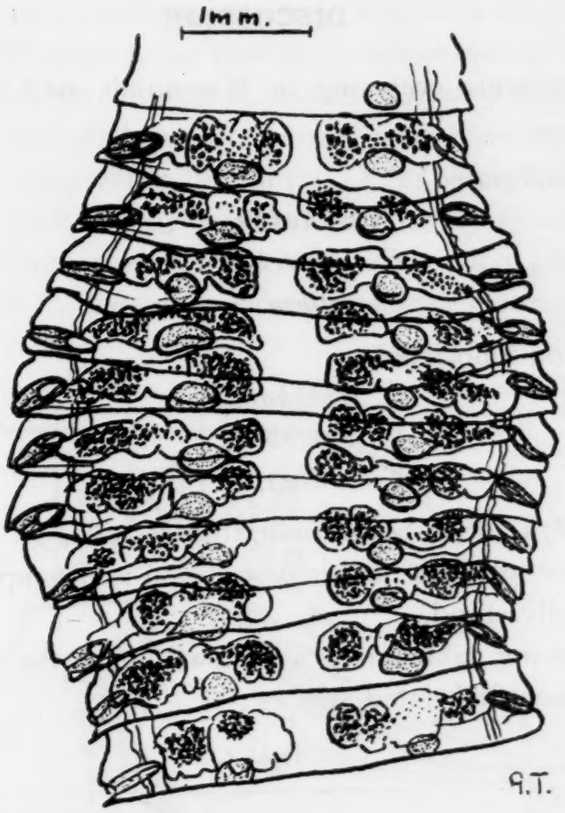

FIG. 5. *C. festiva.* Showing development of uterus.

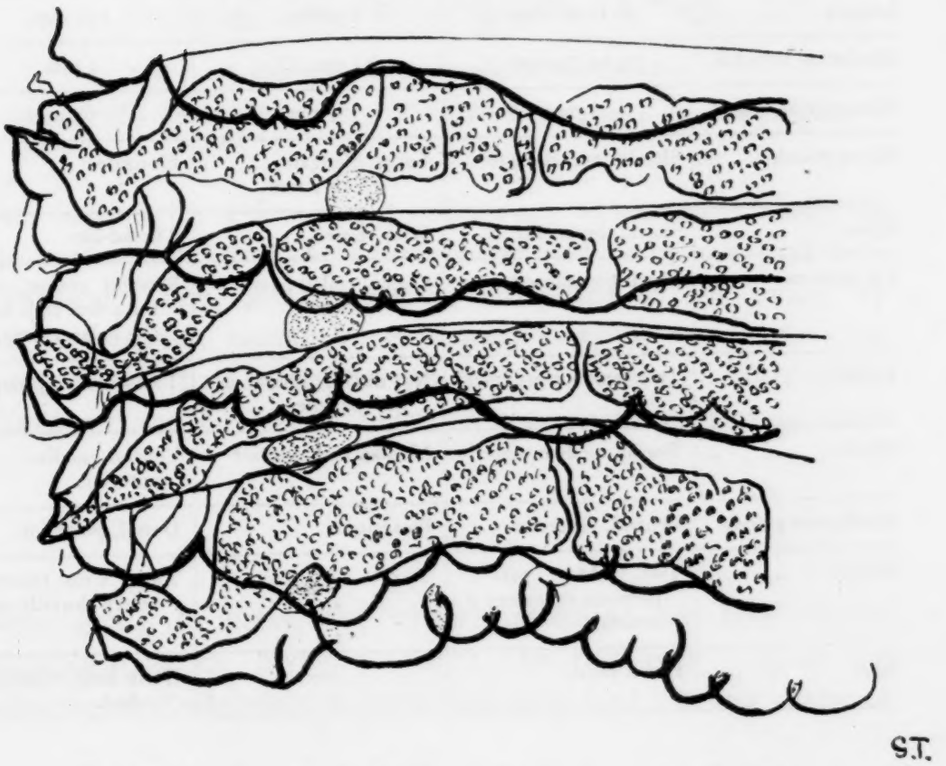

FIG. 6. *C. festiva.* Showing development of uterus; much contracted.

DISCUSSION

The *Cittotaenia* occurring in Marsupials may be divided into two classes :—

(1) Fimbriated :

C. zschokkei,
C. lagorchestis,
C. villosa.

(2) Non-fimbriated :

C. diaphana,
C. bancrofti,
C. festiva.

Krefft (1873) describes a fimbriated cestode, *T. fimbriata*, from a *Haematurus* spp., but as his description is incomplete, his species can now hardly stand.

The following table gives a comparison of the main characters of the three non-fimbriated species :—

Table I.

	C. diaphana	*C. bancrofti*	*C. festiva*
Length	60 to 90 mm.	150 mm.	130 mm.
Maximum breadth	3·5 to 3·9 mm.	14 mm. (?)	5 mm.
Diameter of Scolex	1 mm.	1·9 mm.	0·68 mm.
Cirrus pouch ...	Slender and elongate	Large, elongate, 0·8 to 1 mm.	Elongate.
Cirrus	Short, thread-like	With spine	Thread-like
Vas deferens ...	Much convoluted	Closely coiled near cirrus sac	Straight course, except for a few coils behind the enlargement.
Testes	Two separate groups	Not distinguishable in the specimens	Two separate groups.
Ovaries	Dorsal, median, anterior	Median and anterior	Ventral, median, anterior.
Vitellogene glands	Ventral, posterior	Posterior	Dorsal, posterior.
Uterus	Two. May pass between excretory vessels	Two	Two. Cross excretory vessels dorsally only.
Eggs	Horns short	—	Horns long, tapering coiled.

C. festiva is thus seen to approach *C. diaphana* most closely, the main points of difference being that in *C. diaphana* the vas deferens is much convoluted (fig. 7), and the ovaries occupy a dorsal position, whereas in *C. festiva* there are hardly any convolutions in the vas deferens, and the ovaries lie ventral. For *C. diaphana* the horns of the pyriform body are given as short, whereas in my specimen, which was not quite mature, the horns were long and tapering. *C. diaphana* and *C. festiva* so closely resemble one another in all

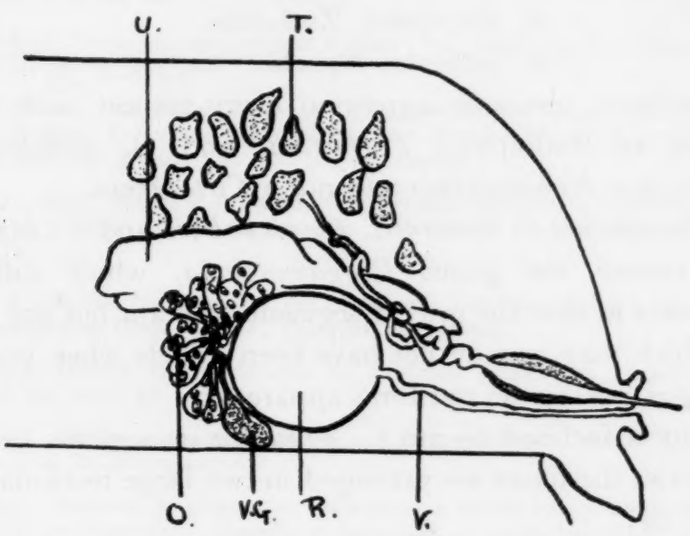

FIG. 7. *C. diaphana.* Mature proglottis after Zschokke.

U.—Uterus; *T.*—Testes; *O.*—Ovary; *V.G.*—Vitellogene gland; *R.*—Receptaculum seminis; *V.*—Vagina.

respects (except for a few slight differences which might easily be due to contraction) that the writer is inclined to believe that they are identical. The dorsal position attributed to the ovary by Zschokke, however, cannot be explained away by contraction; so until more work has been done on the *Cittotaenia* of Marsupials, *C. diaphana* and *C. festiva* must be considered as two separate species.

Since going to press, the writer's attention has been drawn to a publication by Nybelin (1917). In this paper, upon the examination of Rudolphi's original specimen and the new material

at his disposal, Nybelin decides to create a new genus—*Hepatotaenia* for *T. festiva*, Rud. 1819. The genus *Hepatotaenia* differs from the genus *Cittotaenia* in the following respects:—Testes arranged in two groups which do not meet in the median field, and are near to the anterior end of the female genitalia. Vagina opens behind the male genital opening. The two sacciform uteri never fuse with each other.

In this genus he includes:—

H. festiva, Rudolphi.
H. diaphana, Zschokke.
H. fellicola, Nybelin (1917).

The writer's specimen agrees in every respect with Nybelin's description of Rudolphi's *T. festiva*, and is, therefore, to be considered as a *Hepatotaenia* and not a *Cittotaenia*.

For the species *C. bancrofti*, *C. zschokkei* and *C. lagorchestis*, Nybelin creates the genus *Progamotaenia*, which differs from *Hepatotaenia* in that the gravid segments contain but one sacciform uterus, which may or may not have been double when young.

The eggs possess no pyriform apparatus.

Nybelin is inclined to put *C. papillosa* in a genus by itself, as in this species the testes are arranged in two large testicular sacs.

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TWO RARE SPECIMENS OF HUMAN CESTODES

BY

PROFESSOR SADAO YOSHIDA, D.Sc.

AND

SEIJI OGATA, M.D.

Pathological Department, Osaka Medical College, Japan

(*Received for publication* 8 *January*, 1924)

PLATE I

In May, 1923, the writers received two rare specimens of human cestodes from their colleague, Dr. I. Maeda, to whom they had been presented by Mr. Nakasuga, a practitioner in Himeji city, near Osaka. One of the specimens has been identified as *Dibothriocephalus parvus*, Stephens, 1907. Dr. Leon (1915) briefly announced that he had found a case in Roumania. but hitherto the worm has never been reported in Japan. The other specimen is a remarkable malformation of *D. latus*.

1. *DIBOTHRIOCEPHALUS PARVUS*, Stephens, 1907

The present specimen was obtained in August, 1922, from a boy aged nine years, the son of a restaurant proprietor in Himeji city. The patient had been in the habit of eating fish from various parts of the country. He had never left his native home except on a trip to Osaka, in the spring of last year.

The strobila without the head measures 2·315 metres in length, and consists of about 1,150 proglottides. During the nine months that had elapsed since the expulsion of the worm, the patient had not shown any sign of the presence of a parasite either in the faeces or in the way of symptoms. Hence it is probable that the head was passed with the strobila, but was overlooked.

The anterior segment is 0·55 mm. broad and 1·0 mm. long, the posterior segment 5·5 mm. broad and 3·0 mm. long, the maximum

segment being 7 mm. broad and 3 mm. long, and occurring six to ten centimetres from the posterior end. The segments of the strobila gradually increase in breadth and in length posteriorly; the former dimension is always greater than the latter, except in a few segments at the anterior extremity which are longer than broad, probably because of the elongated state of the worm. This regular increase in the dimensions of the segments towards the posterior end is interrupted here and there by the irregular extensions and contractions of the segments. The segments, 100 cm. behind the anterior end, are approximately quadrate in shape, both the length and breadth being 3·5 to 4·0 mm. Actual measurements of some segments at various distances from the anterior extremity are as follows:—

	Breadth	Length		Breadth	Length
20th Segment ...	1·5 mm.	1·0 mm.	205th Segment ...	2·6 mm.	0·6 mm.
500th Segment ...	3·5 mm.	2·0 mm.	623rd Segment ...	4·0 mm.	3·5 mm.
1000th Segment ...	6·0 mm.	3·0 mm.	1030th Segment ...	7·0 mm.	3·5 mm.

(The number of the segments given above does not indicate the first segment of the corresponding breadth and length; for instance, the 20th segment is not necessarily the first one of the segments which are 1·5 mm. broad and 1·0 mm. long.)

The posterior border of each segment overlaps the anterior border of the segment following it, and consequently, the lateral margins of the worm present a serrated appearance which differs a little from the description given by Stephens. The surface of the worm is much wrinkled with transverse and longitudinal furrows. The state of corrugation varies according to the condition of the contraction of the segments; thus, in the most extended segments, the transverse wrinkles disappear although the longitudinal ones still exist, while in the contracted segments the transverse and longitudinal furrows are numerous and most conspicuous. Throughout the entire strobila, two (four in all) distinct longitudinal furrows run almost uninterruptedly along the submedian lines on both ventral and dorsal surfaces. Probably these continuous longitudinal furrows are situated on the lines corresponding to the

lateral nerve cords; they lie at a distance of 2 mm. from the lateral margins of a segment 7 mm. wide.

The cirrus opening is situated about one-third to one-fourth of the length of the segment from the anterior margin; the uterine openings cannot be easily made out with a hand-lens owing to the distortion caused by wrinkles on the surface.

The yellowish mass of the uterine loops can be recognised by the naked eye 50 cm. from the anterior end of the worm. As the uterine tube enlarges, the loops form yellowish globular tubercles upon the dorsal surface. Under the microscope, the uterus appears as a central rosette with four to six loops on each side.

The longitudinal layer of the parenchymal musculature is diffuse and more weakly developed than that of *D. latus*, which consists of well developed muscle-bundles.

The testes are very irregularly in the medullary field, either in a single row or in two rows, one of which is directly or obliquely ventral to the other.

The eggs are operculated, oval in shape, similar to but slightly smaller than those of *D. latus*, measuring on an average 58μ in length and 39μ in breadth; the lengths varying from 52·7μ to 67·3μ, and the breadths from 36·5μ to 41μ.*

From the above description, it is clear that our specimen belongs to the species *D. parvus* Stephens. Stephens gave five special characters as distinctive of his species, and the writers incline to concur in his conclusions. His main distinction is, however, based upon the external characters, especially the size of the worm and of the eggs. When identifying parasites, the size of the worm and of the eggs alone is not always sufficient to distinguish the species. In the course of his work on *D. mansoni*, Yoshida found that the adult form of this species varies greatly in size according to the host or other conditions of parasitism. The adult worms obtained from dogs fed with the liguloid larvae from the human host and from snakes measure 2 to 3 metres in length, while the worms from young cats fed with similar liguloid larvae from snakes and frogs are very small, measuring only 30 to 40 cm. in length. During recent years, Japanese investigators have shown experimentally that the tape-

* The writers propose in another paper to compare the internal anatomy of the closely related species, *D. latus*, *D. decipiens* and *D. mansoni*, in regard to which some confusion exists.

worms developed from the liguloid larvae of human beings, frogs and snakes are all the same species, namely, *D. mansoni.* This identification, however, is based chiefly upon the developmental study and external morphology of the worm, and not upon the details of internal structure. Thus, much work remains to be done on the internal morphology of the parasites in order to distinguish *D. latus* from *D. parvus*, and to determine finally whether the liguloid larvae of man, frogs and snakes develop into one and the same species or not.

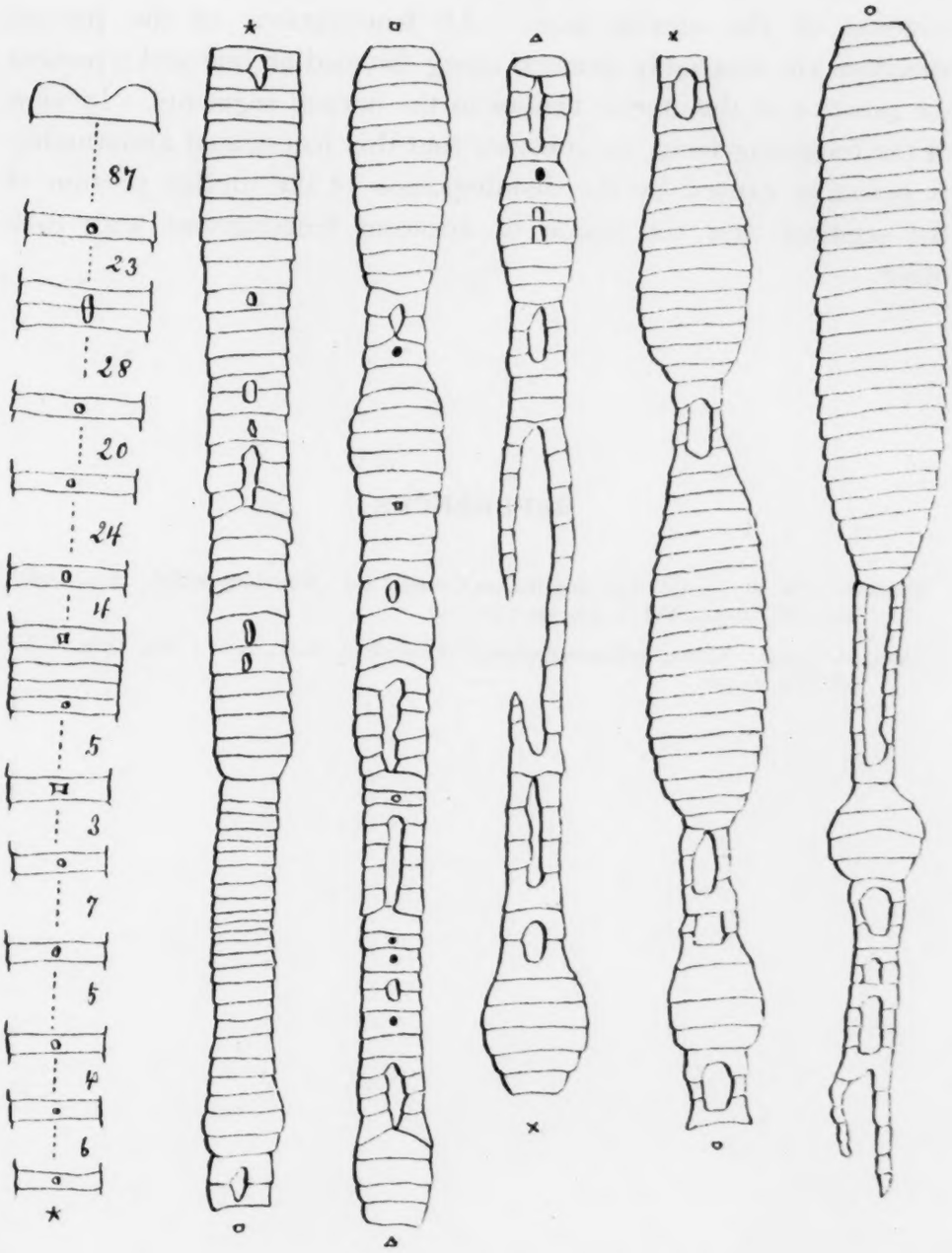
Some Japanese authors are of opinion, from a study of the liguloid larvae, that *D. mansoni* may be identical with *D. decipiens* of the cat. The two forms, however, have not yet been absolutely identified, as a precise description of *D. decipiens* is lacking, especially as regards the size and shape of the eggs. For the same reason, it is not possible to make a satisfactory comparison of *D. decipiens* and *D. parvus*.

2. MALFORMATION OF *DIBOTHRIOCEPHALUS LATUS*

This abnormal specimen was also presented by Mr. Nakasuga, who obtained it from a man aged about 50 years. It is a piece of strobila of 1·1 metres in length, proglottides being four hundred and eighty-eight in number, and the maximum breadth 13 mm. It presents remarkable abnormal segments, especially numerous in the posterior half of the specimen. The posterior end is bifurcated, each limb consisting of six segments. A few wedge-shaped segments are found in the anterior part of the strobila. The most pronounced abnormality is the fenestration which occurs in over forty segments. The largest one is 34 mm. long and stretches over ten segments, one side of it being discontinuous. The second largest fenestration is 22 mm. long covering eight segments, the third 9 mm. long over four segments; then come two fenestrations 8 mm. long over five segments, four 6 mm. long over four segments, five 5 to 5·5 mm. long over three segments, four 3 to 4 mm. long over two or three segments; the others are all rather smaller and are situated on a single segment or between two segments (fig. 1).

In some segments, the uterine loops become enormously enlarged to form a globular tubercle on one side of the worm, while the

FIG. 1.



Semidiagrammatic figure × 3/2. Figures between the segments on the left denote the number of segments without a perforation.

corresponding portion on the other side presents a depression of varying depth. In other segments, the depression becomes deeper and deeper and ultimately causes the fenestration, owing to the removal of the uterine mass. All fenestrations of the present specimen are invariably situated along the median line and represent the position of the uterine masses in the normal segments. In view of the foregoing facts, we consider that this fenestrated abnormality is probably caused by the disintegration of the uterine portion of the segment and the fusion of adjacent fenestrations with each other.

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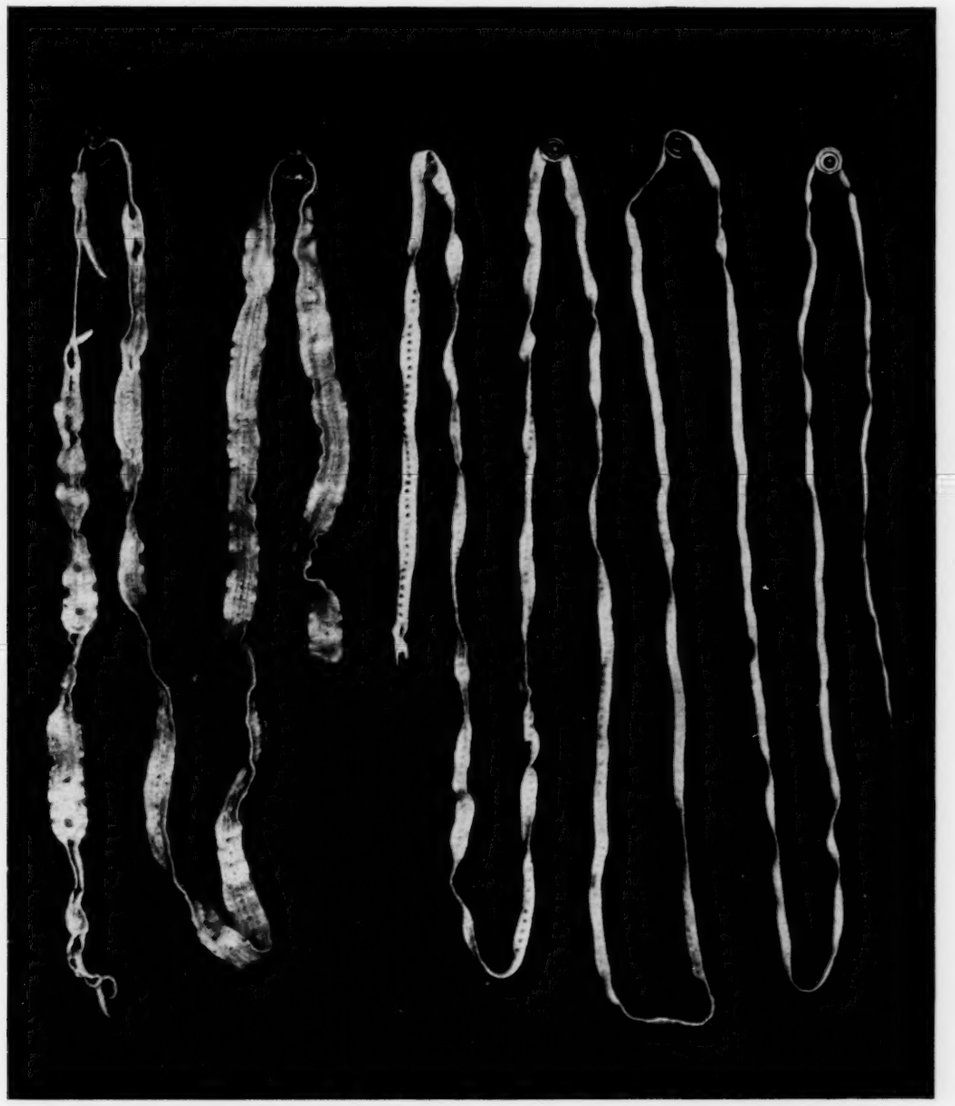
EXPLANATION OF PLATE I

Left. *Dibothriocephalus parvus*

Right. Malformation of *Dibothriocephalus latus*

PLATE I



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A PIG NEMATODE, *GNATHOSTOMA HISPIDUM*, FEDCHENKO, AS A HUMAN PARASITE

BY

K. O. R. MORISHITA

Department of Medical Zoology, The Kitasato Institute for Infectious Diseases, Tokyo, Japan

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Recently Dr. M. Kinoshita, of the Surgical Clinic of the Tokyo Imperial University, brought to me for identification a small piece of fresh human cutaneous tissue containing a parasite. Unfortunately, the nematode, which I was able to remove easily from the tissue, had been mutilated, apparently during the operation. There still remain, however, enough points of systematic importance to enable me to identify it with certainty as *Gnathostoma hispidum*. At least four cases of *Gnathostoma* in man have hitherto been reported, but they are all referred to *Gnathostoma spinigerum*, Owen. This paper presents, therefore, the first case of *Gnathostoma hispidum* in man.

Gnathostoma hispidum is a parasite generally found lodged in the stomach of wild and domesticated pigs, and has been found only once in the fat layer of a cow, in Berlin. In Japan, excluding Formosa, there have come to my knowledge only five cases of the occurrence of this species in the stomach of the domesticated pig, and this is the only species of *Gnathostoma* hitherto found in this

country. The new case reported here is of especial interest, not only because it concerns *Gnathostoma hispidum* as a human parasite, but also on account of its pathogenic properties.

PATHOGENICITY

The patient is a male Japanese, 43 years old, who has lived long in Tokyo. On the evening of May 12th, 1923, he suddenly felt a slight pain on the left thenar eminence. He found a linear swelling of about 1 cm. there, and its length increased during the same and the following days to about 5 cm., with continued pain. On the third day the length of the swelling was about 8 cm. The patient then consulted Dr. Kinoshita, who recognised typical clinical features of the creeping disease, *Dermatitis linearis migrans*, in the affected part, and at the end of the progressive linear swelling, a small black object through the skin. On the same day Dr. Kinoshita excised the affected region to the length of about 2 cm., including the black object, and quickly brought it to me. This black object proved itself to be the intestine of the parasite, containing blood probably derived from the host. After the operation the symptoms disappeared. The worm was, therefore, the cause of the creeping disease.

DESCRIPTION OF THE WORM

The worm is a young female. The head and anterior body wall are lacking. The posterior part of the body is cylindrical, tapering very gradually posteriorly to the abruptly rounded end. From the anterior end of the oesophagus to the posterior end of the body is about 5 cm., and the broadest part of the portions of the body which remain measures 0·51 mm. There are many fine transverse striations and small annulations on the cuticle, at intervals of 22μ to 33μ. Small spines cover the whole body. The rows of the spines do not always agree with the annulations of the cuticle, as in the anterior part the intervals between the spine rows are 7μ to 10μ. The spines are simple and directed posteriorly. In the anterior region

they measure 6μ to 7μ long by 2·4μ to 3·6μ broad at the base and become smaller posteriorly, measuring in the hindmost part only 3·6μ long by 2·4μ broad at the base, although large ones may occur at times.

The rows of spines are generally close together anteriorly and more separated posteriorly, and the spines of the same row are correspondingly more separated posteriorly, until finally they no longer form rows but are scattered irregularly and appear as dots. Ventrally the spines extend to about 74μ from the posterior end, and dorsally more close to the latter. The lateral lines are apparent, and the somatic muscle cells are spindle- or tadpole-shaped.

The one cervical sac which has fortunately been left is cylindrical, measures 0·4 mm. long by 50μ broad in the distal region, and is slightly constricted at 0·125 mm. from the distal end. A narrow duct is seen running through the entire sac.

The oesophagus is thick and club-shaped, measuring about 1·2 mm. in length; its maximum breadth, which lies in the posterior region, is 0·027 mm. Its lumen is lined by a triradiate cuticular wall. The intestine is of about equal breadth throughout, and only narrows slightly in the most posterior part. Its breadth is 0·135 mm. at the commencement, 0·22 mm. at the broadest point lying before the middle, and 0·115 mm. in the posterior end. The wall of the intestine consists of pentagonal or hexagonal epithelial cells. The rectum is conical and about 0·15 mm. long. The anus lies at the distance of 0·08 mm. from the posterior end of the body. Owing to shrinkage, the tail is seen as a conical process. As to the genital organs I have nothing to note.

The foregoing is all that I have been able to ascertain in my specimen. Although it is incomplete, the presence of cervical sacs and the mode of arrangement of the body spines conclusively prove it to be *Gnathostoma hispidum*.

I have still two other cases to note, which also refer to *Gnathostoma* in man. Both are from China. With the four generally known ones we can, therefore, count seven indubitable cases of *Gnathostoma* in man, and all are restricted to Eastern Asia, *i.e.*, Siam, Malay States, China and Japan. The cases from China will be reported on in another place.

CONCLUSIONS

1. A nematode newly recovered from man in Japan is *Gnathostoma hispidum*, Fedchenko.

2. This is the first recorded case in which this species occurs as a human parasite.

3. The worm caused a typical creeping disease.

I am indebted to Professor S. Goto for his kind supervision, and to Dr. M. Kinoshita for kindly placing the material at my disposal; my sincerest thanks are due to both of these gentlemen.

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GASTRODISCUS HOMINIS

BY
CLAYTON LANE

(*Received for publication* 14 *January*, 1924)

Under the heading '*Gastrodiscoides hominis*,' Leiper (1923) wrote thus: 'The systematic position of this parasite, found first by Lewis and McConnell in 1876, has been the subject of consideration by several workers. Placed in the genus "Amphistoma" by the discoverers, Fischoeder, in 1902, on account of the flattened ventral aspect, transferred it to the genus *Gastrodiscus*, and in 1913 Leiper made it the type species of a new genus *Gastrodiscoides*. The validity of the separation of the species from other forms, *G. aegyptiacus* and *G. secundus*, met with in the horse in Africa and India respectively, has been questioned by Lane (1922), but has been supported more recently by Khalil (1923).'

The characters of *G. hominis* selected by Leiper (1913) as justifying its generic separation were:—

(1) Large genital cone.
(2) Position of genital orifice.
(3) Smooth ventral disc.
(4) 'Testes tandem.'

The reported examinations of *G. hominis* are the following:— Lewis and McConnell (1876) dissected specimens obtained by autopsy from man; Stephens (1906) examined by section material collected by Ross in Assam; Leiper (1913) reported examination by section of material collected from man by Wise in British Guiana, and by Mackie in Assam; Lane made complete transverse and sagittal serial sections of worms from Lewis and McConnell's original material received through the courtesy of Sir Leonard Rogers, in effect type material, although no type specimen had been designated by Lewis and McConnell. With Low (1923), he then reported in condensed form the results of his reconstruction, having, as a preliminary, demonstrated one of the sections at a laboratory meeting of the Royal Society of Tropical Medicine and Hygiene

(Lane, 1922); Khalil (1923) described material and illustrated a squash preparation harboured by the Napu mouse deer (' *Tragulus napu*') from the Malay States, which died in the gardens of the Zoological Society of London; Maplestone (1923) re-examined Stephens's sections.

These descriptions have dealt with Leiper's four generic characters as follows:—

(1) Stephens makes no mention of a genital cone. In Leiper's specimens it was markedly present. In Lewis and McConnell's collection there was, as Lane's sections showed (Lane and Low, 1923), no cone but a depression surrounded by a tuberculated area encircling the opening of the genital atrium, a structural arrangement to which Lane (1923, p. 1693) gave the designation of genital pro-atrium. At this spot, as was indicated, the subcuticular sheet of muscle is incurved as a dome surmounting the genital pore; this on contraction and flattening would clearly evert the genital pro-atrium and produce a genital papilla. This dome of muscle, equally marked in longitudinal or transverse serial sections, Lane designated as a pseudo-sucker. He emphasised the fluke's affinities with *Watsonius*, already noted by Leiper ten years earlier, by placing both genera in a new tribe Gastrodiscidi, one of whose features was this same muscular dome, which Stiles and Goldberger (1910) had described for *W. watsoni* as 'a well-defined, curved (with convexity dorsal), muscular layer.'

Khalil distinguished a genital cone in all the fifty specimens he examined, and considers Leiper justified in taking a cone as one of his differentiating points for this genus. No amount of negative evidence, however, can invalidate a single positive observation; no matter how often a genital depression may be absent, its authenticated presence eliminates it inevitably as a valid generic character; and a genital depression is a marked feature of Lewis and McConnell's 'type' material.

Maplestone (1923) next gave two illustrations of Stephens's material. In one fluke there is a marked genital papilla, in the other there is none. Further, he illustrates *Cotylophoron cotylophoron*, *Brumptia gigas* and *Paramphistomum explanatum*, showing how, in each case, the genital opening may lie at the summit of a papilla or at the bottom of a depression, and writes of *G. hominis*,

'These two drawings, taken in conjunction with Leiper's figure, indicate that the presence or absence of a prominent genital papilla, or of a genital atrium, are purely matters of chance, and are of no more diagnostic value in this instance than in any other species of the group *Amphistomata*.'

The evidence, therefore, is that *G. hominis* may, in common with other Amphistomata, show either a genital papilla or a genital depression, and that it possesses certain anatomical muscular dispositions clearly capable of producing these alterations in the fluke's profile.

(2) Leiper does not elaborate this generic distinction by which *G. hominis* differs from other members of the genus *Gastrodiscus* beyond saying that it lies in 'the nearness of the genital pore to the edge of the ventral disc-like expansion.' It may be considered in greater detail. In *G. hominis* this pore lies about the middle of the anterior portion; in *G. aegyptiacus* about the anterior margin of the disc; in *G. secundus* well within the disc. *G. minor*, the other species of this genus, is practically a *nomen nudum;* its original designation (Leiper, 1913) never having been amplified; Maplestone treats it as a synonym of *G. aegyptiacus*, and it need not further enter into these considerations. But in *G. hominis* where does the disc begin? Stephens noted that neither anteriorly nor posteriorly did his specimens have a complete rim to the disc. Leiper characterised this statement as not accurate, since the rim 'always is sufficiently well marked to distinctly separate off the anterior conical portion of the worm from the disc.' Of the three specimens which Lane illustrates, the anterior rim of the disc is ventrally marked in one, indefinite in one, absent in one. Khalil found the distinction between disc and cone indefinite. But if the anterior margin of the rim be ventrally an inconstant structure, the line of junction of cone and disc may be roughly established by the lateral and dorsal aspects. Thus judged, Lane illustrates in Lewis and McConnell's material the anterior portion as occupying 3·5 out of 7 mm.; Khalil as 2 out of 7 mm. The anterior edge of the disc is then a point of varying position and particular indefiniteness in *G. hominis*, and one not affording a basis for generic distinctions. Moreover, if the position of the genital pore be considered relative to the viscera, if in accordance with modern usage the internal

anatomy be substituted as a systematic basis for the unsatisfactory one of external appearance, the pore in *G. aegyptiacus* and in *G. hominis* lies about opposite to the middle of the oesophagus, in *G. secundus* posterior to the oesophageal bifurcation. It would not be more arbitrary to suggest this as a generic character than the one based on the superficial shape, but Maplestone has shown that the position of the genital pore in front of or behind the gut fork is not even of specific value in this group.

(3) No one has examined the prominences found on the ventral surface of the disc of *G. aegyptiacus* so minutely as Looss. He described, in addition to the ordinary body musculature and beneath and parallel to the ventral surface of the disc, a network of very strong muscular fibres, the strands crossing at right angles and interlacing where they cross. He points out that general contraction of the fluke will force the parenchyma through the meshes so formed, and that local contraction of the fibres is to be looked upon as capable of producing a like effect. There exists, then, an anatomical disposition of muscle, and upon that disposition and upon muscular contraction does the presence of these eminences depend. They are prominent or replaced by depressions—with intermediate stages—according to the general and local state of muscular contraction.

It scarcely requires reiteration that neither genus nor species can rest on shape induced by muscular contraction.

(4) 'Testes tandem.' Stephens has shown that what Lewis and McConnell judged to be the ovary was really the posterior testis. The original observers accordingly found the testes tandem, as did Stephens, Leiper and Lane. In Khalil's material, surely preserved under ideal conditions, the illustration of a squash preparation, which he accepts in his description as generally applicable, shows them strictly diagonal. The first writers used dissections, the next three reconstructed serial sections, the last both squashed and sectioned flukes. Furthermore, in the illustrations of Lewis and McConnell, Stephens, and Khalil, both testes are in the disc; in those of Leiper the anterior testis lies at the junction of the two portions, but extends further into the cone than into the disc; in Lane's, the anterior testis is far in the cone if one judge by the dorsal margin; in the disc, if one judge by a possibly significant ventral depression, it being a matter of

arbitrary personal opinion as to whether it be judged to lie in cone or disc. Khalil considers 'that Leiper's definition of the genus ought to be modified as regards relative position of the testes.' The position, however, seems to be as follows:—The anterior testis may in undistorted specimens lie in the disc or more or less in the cone, the other testis lying axially behind it; in squash preparations the other testis may readily be made to take an oblique position. In other words, their relative position is alterable with methods of examination, and cannot be made a generic character.

It appears, therefore, that the genital papilla, the ventral prominences, the relation of the genital pore to the anterior edge of the disc, and the position of the testes are data insufficient to justify the creation of the genus *Gastrodiscoides*.

The work of Maplestone (1923) on this group, by the mass of observed fact on which it is based, stands apart from all that has gone before. Where others have proposed a new species or genus upon a single specimen, not infrequently immature, he has shown by the examination of hundreds that full gradations exist between different supposed species so that but one exists, and has made a very weighty addition to the existing protest against the use of the shape and size of muscular structures as of specific or generic value. He has shown, among other things, that the generic differences which induced the separation of *Watsonius* from *Pseudodiscus* are, on analysis, untenable, so that the valid name of man's parasite will become *Pseudodiscus watsoni*. But the considerations which he adduces, based on these hundreds of examinations of various species, cannot evidently be limited to the group concerned; they must have a general applicability for trematodes. For instance, the specific differences between *Euparyphium malayanum* and *Eu. suffrartyfex*, in so far as they refer to the shapes of the flukes and, it may be added, to the relative sizes of the testes, are untenable, and the validity of the latter species will depend upon whether the number of necklet spines and the presence of large corner spines prove in this genus to be true unvarying specific characters.

Similarly his considerations show that while Leiper was correct in refusing to accept the effects of muscular action as a valid basis for Stephens' *Paropisthorchis*, he was incorrect and inconsistent in accepting them for *Gastrodiscoides*. They justify the already

advocated suppression of *Watsonius macaci*, Kobayashi, 1920, and *Heterophyes nocens*, Onji and Nishio, 1915, as synonyms of *W. watsoni* and *H. heterophyes*, respectively (Lane and Low, 1923). They must equally be used to eliminate those cestode species, in so far as they are based on overlapping measurements, whose diagnosis is such that it may be a mere matter of personal taste as to which species shall have the credit of harbouring an individual specimen.

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SOME MORPHOLOGICAL FEATURES OF *PLASMODIUM FALCIPARUM*

BY

J. W. W. STEPHENS

(*Received for publication 22 January*, 1924)

PLATES II, III

W.S. admitted to hospital 7.10.22 from West Africa.

A microscopical examination of stained films (Leishman or Giemsa) showed numerous *P. falciparum* parasites and a very scanty crescent infection.

The red cell infection rate was approximately 1 per cent., and the relative numbers of single and multiple infections were :— single, 69 per cent. ; multiple, 31 per cent.

The large majority of the parasites were uniform in size, of a diameter approximately equal to a third of that of the red cell. No pigment was detected in any of these ' rings.' It was frequently observed that part of the cytoplasm stained a deep bluish black, either in the form of an isolated spherical dot or dots, resembling somewhat pigment grains, or in a streak not uncommonly extending from the nucleus ; this shade of colour contrasting sharply with the lighter blue of the rest of the parasite. The form of the nucleus, while variable, was commonly that of a half hoop or bar.

A more prolonged examination of these films revealed many peculiar forms, some of which are here figured diagrammatically.

These forms were somewhat uncommon, but could always be found by systematic search through the films with a mechanical stage.

They can be arranged in three groups.

PLATE II—

(*A*). *Those in which there is a cytoplasmic connection between two adjacent parasites.*

Figs. 1-4. The appearance is that of a bridge or strand varying in width, in some cases clearly visible ; in others (not figured) the apparent strands were so thin that doubt arose as to their reality.

(*B*). *Those in which there is a nuclear connection between two adjacent parasites.*

Figs. 5 and 6. The parasites overlap, and accordingly, the appearance of a nuclear connection may be accidental.

Fig. 6. The symmetry of the two unusually elongated parasites is noteworthy.

Figs. 7-9. Fine threads pass from one nucleus to the other.

Fig. 8. Whereas in Fig. 7, two parasites were evidently present, in this case it was impossible to be certain whether one or two separate parasites existed.

Fig. 9. Has the appearance of a dividing parasite, the red cell being spindle-shaped. No other example like this was found.

Figs. 10-16. Thicker easily visible strands stretch between the nuclei.

Fig. 10. In this case again, apparently only one parasite body was present, and the nuclear symmetry is striking.

Fig. 12. A parasite beginning to divide or a parasite with a bilocular ' vacuole.'

Figs. 13, 14, 16. The parasite bodies are distinctly separated from one another in each case and apparently are in process of division.

Fig. 15. Traction may have had an influence in producing this extended form.

PLATE III—

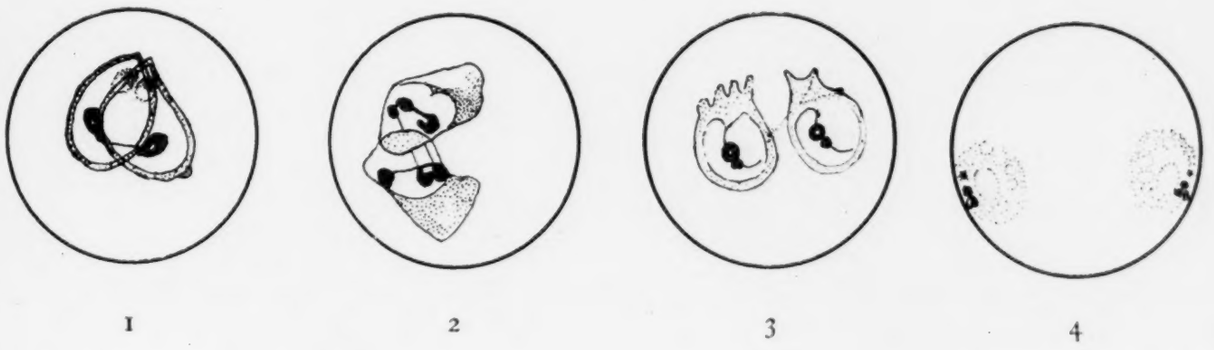
(*C*). *Symmetrical Parasites.*

Figs. 1-4. Although speaking generally, one ' ring ' is like another, in these cases the symmetry and correspondence in morphological detail would appear to be more than an accidental occurrence.

(*D*). *Miscellaneous forms.* These include a number of forms differing considerably in appearance from those described in the standard text-books.

Fig. 5. Has two symmetrical nuclei, a rare feature in the parasites in these films.

Fig. 6. A very peculiar form. Two overlapping parasites, one having a curved crescent-like appearance.



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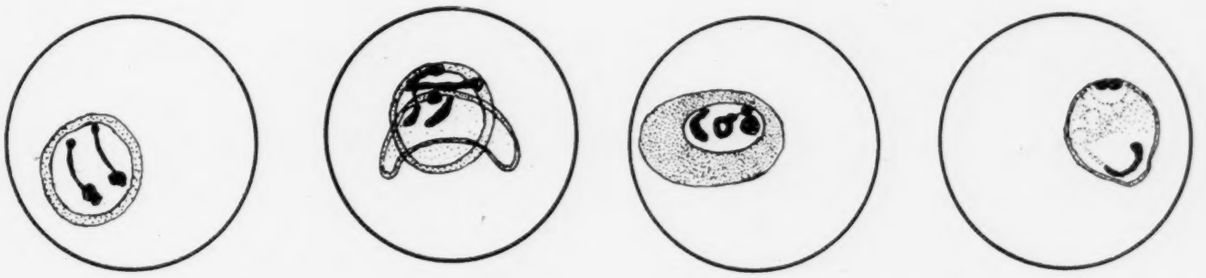
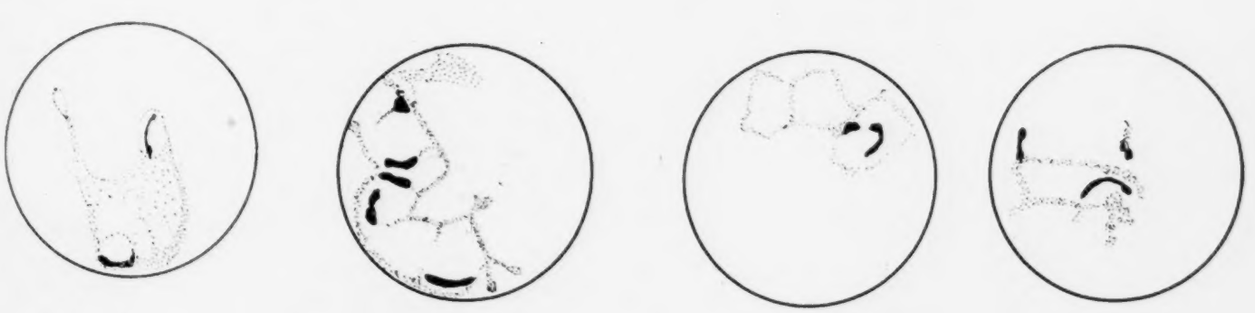


5 6 7 8

9 10 11 12



13 14 15 16

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Fig. 7. A large parasite form, staining intensely, with several nuclear masses, very unlike other ' ring ' forms.

Figs. 8-13. It is impossible to say in each case how many parasites are present.

Figs. 11 and 12. The appearances suggest plasmodial masses.

Fig. 13. The sharp-tailed ends are noteworthy.

Figs. 14-16. Extraordinarily irregular forms, in which it is impossible to distinguish how many parasites are present. Whether these forms should be called ' disintegrating ' or ' degenerating,' etc. seems questionable without further evidence.

THE PEARL-INDUCING WORM IN THE CEYLON PEARL OYSTER

BY

T. SOUTHWELL

(*Received for publication* 26 *January*, 1924)

The Ceylon Pearl Fisheries are characterised by the fact that the harvest obtained from them is of a very irregular character. Occasionally, fisheries have been held every year for a number of years in succession, but the historical record of the pearl banks shows that numerous barren years, sometimes extending over long periods, have intervened.

As a pearl fishery yields considerable revenue to the Ceylon Government, the Colonial Office decided in 1900 that a thorough examination of the conditions existing on the Ceylon Pearl Banks should be made, the object being to ensure annual fisheries, if possible. On the recommendation of the Council of the Royal Society, Professor W. A. Herdman, F.R.S., accompanied by Mr. James Hornell as assistant, proceeded to Ceylon in December, 1901.

After an examination of the Pearl Banks, Professor Herdman returned in April, 1902, but Mr. Hornell remained in Ceylon to carry on the work.

In 1906, the Ceylon Company of Pearl Fishers leased the Pearl Fisheries from the Government, and the writer went out to Ceylon as Scientific Officer ; on the resignation of Mr. Hornell in 1907, he became Scientific Adviser and Inspector of Pearl Banks to the Company.

In 1908, Professor Herdman paid a short second visit to Ceylon, spending practically all his time on the Pearl Banks.

The Ceylon Company of Pearl Fishers ceased to exist in 1912, and the writer resigned his post in November, 1911.

The general work done by these officers and the results arrived at are not material to this paper, except in so far as they relate to pearl formation and the pearl-inducing worm.

Before proceeding to a discussion of this problem it is desirable to point out that pearls may be differentiated into three kinds, viz. :

(1) EXCRESCENCES or BLISTERS on the inside of the shell, caused by boring animals or other foreign bodies. This type of ' pearl ' will not be considered further in this paper.

(2) MUSCLE-PEARLS or SEED-PEARLS. Small irregularly shaped pearls, usually occurring under the epidermis in ' the region where the muscle-attachment epithelium passes over into the ordinary shell-secreting epidermis of the mantle' (Jameson). These seed- or muscle-pearls were presumed by Herdman and Hornell to be formed round minute limy concretions which were called calcospherules. Seed-pearls are usually numerous, often in clusters, small, irregular in shape and situated in the vicinity of the muscle insertions.

(3) CYST PEARLS or ORIENT PEARLS (the valuable pearls of commerce). Herdman and Hornell believed that in the majority of cases cestode larvae formed the nucleus of Orient pearls in the Ceylon Oyster, and that the adult form of this larva was *Tetrarhynchus unionifactor*, Shipley and Hornell, 1904, although Herdman later on stated that ' Cestodes, Trematodes and Nematodes are all concerned in pearl-formation.'

In order to understand the nature of the problem it is necessary to point out that the oyster shell is structurally composed of :—

(1) An outer layer called the Periostracum.

(2) A prismatic layer.

(3) The nacre, or mother-of-pearl, forming the bulk of the shell as well as the internal lining.

(4) The hypostracum, a substance secreted by a specialised epithelium and by which the muscles are attached to the shell.

(5) The hinge ligament.

The periostracum and the prismatic layers are secreted from the edge of the mantle, whilst the nacre, or pearly layer, originates from the whole of the outer surface of the mantle. The ligament is continuous with the periostracum.

The oyster is commonly infected with three principal larval parasites, viz.,

(1) A larval *Tetrarhynchus*, found in the wall of the gut (figs. 1 and 2).

FIG. 1. Older larval stage of *Tetrarhynchus* met with in the tissues of the pearl oyster's gut. After Shipley and Hornell. × about 12.

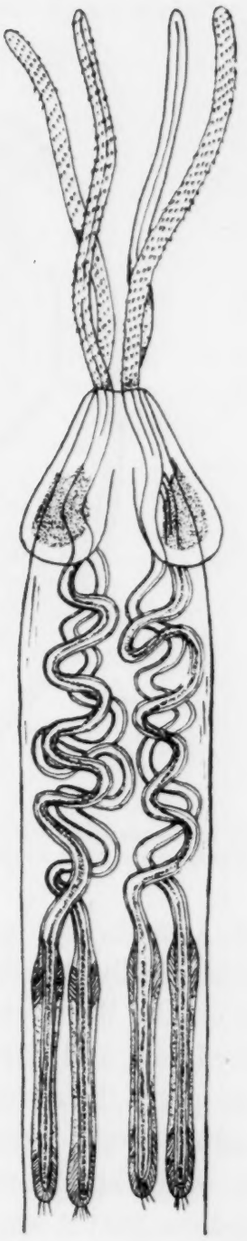

FIG. 2. Same, more highly magnified. After Shipley and Hornell. × about 50.

(2) A rather large (0·5 to 1·5 mm.) globular Cestode larva morphologically belonging to the genus *Tylocephalum*, found in the liver, etc. (fig. 3).

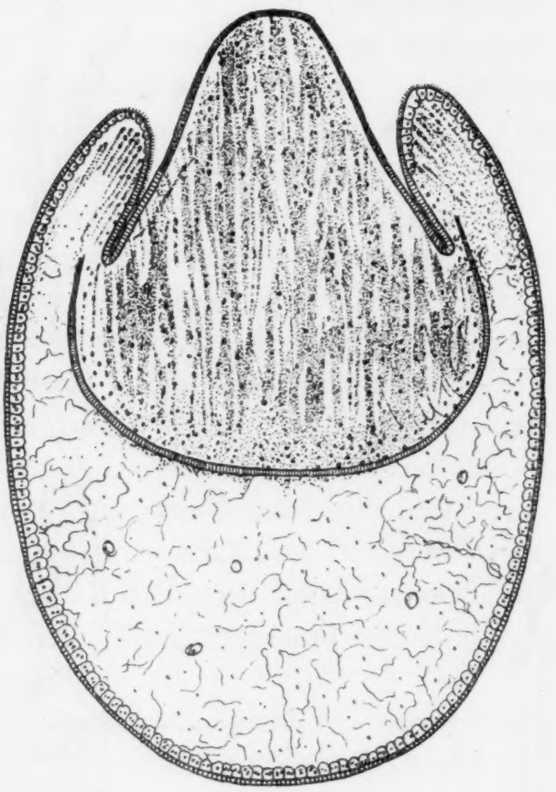

FIG. 3. Longitudinal section through the globular larva of *Tetrarhynchus unionifactor*, Shipley and Hornell, 1904. After Southwell. × 960.

(3) A smaller (about 0·1 mm. to 0·2 mm.) globular Cestode larva, morphologically belonging to the genus *Tylocephalum*, and also found in the liver, etc.

Herdman and Hornell believed that the *Tetrarhynchid* larva was simply a later stage of the larva in the globular cysts and that the adult worm occurred in different species of Elasmobranch fishes, which are known to feed on oysters. Herdman, however, pointed out that it was possible that the globular larvae (Nos. 2 and 3 above) might belong to the genus *Acrobothrium* (= *Tylocephalum*). The adult *Tetrarhynchus unionifactor* has not, up to the present, been adequately described.

The distinction between these two types of larvae was clearly recognised by the present writer, who in 1910 wrote ' it would

certainly appear more probable as well as simpler for this larva (Nos. 2 and 3 above) to develop into a *Tylocephalum* (as is believed by Seurat) than into a *Tetrarhynchus*.'

The wide difference between the genera *Tylocephalum* and *Tetrarhynchus* will be evident from figs. 4 and 5.

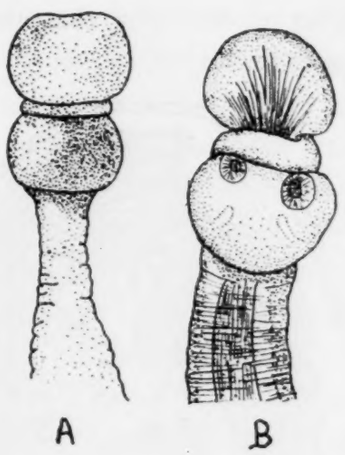

FIG. 4. *Tylocephalum pingui*, Linton, 1890. *A*—Head and neck of living specimen. × 18. *B*—Same when made transparent in clove oil. × 24. After Linton.

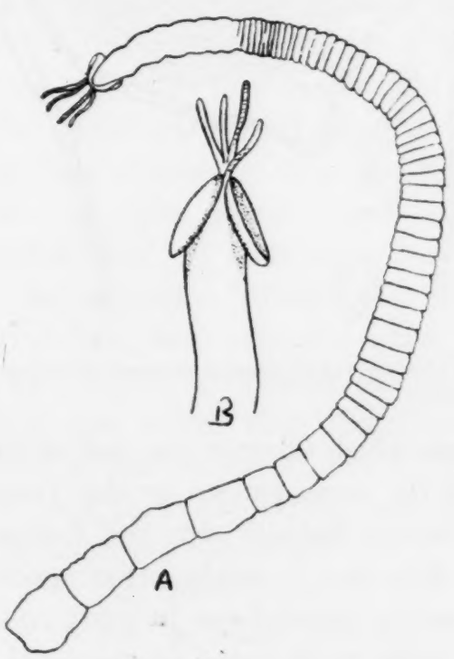

FIG. 5. *Tetrarhynchus unionifactor*. *A*—Entire worm. × 8. *B*—Drawn from life, showing fusion of the bothridia anteriorly, and the apical emergence of the proboscides. × about 25. After Shipley and Hornell.

A cyst pearl is almost invariably formed round a nucleus. This nucleus is believed to set up local irritation which results in a migration of epithelial cells normally concerned in secreting the nacre of the shell to the offending particle which it surrounds as a globular pearl-sac. The particle is thus coated with successive globular layers (fig. 6). It is believed that pearl formation only takes place round larvae which have died for reasons unknown, and which accordingly set up local irritation.

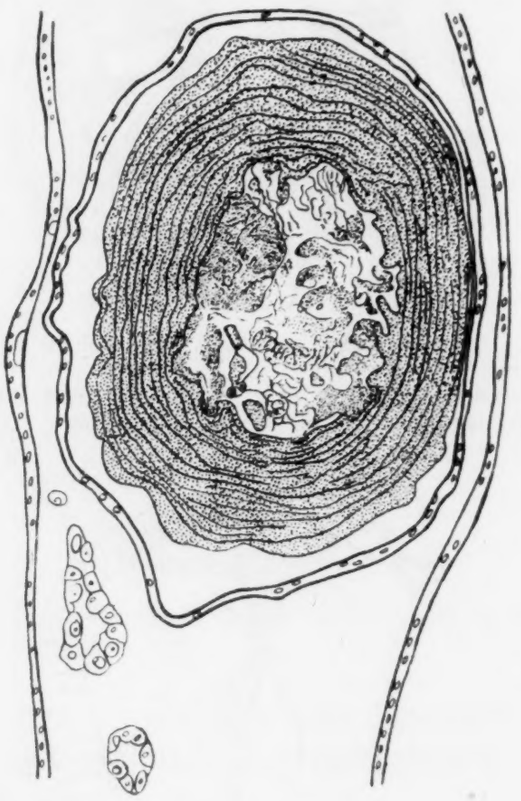

FIG. 6. Pearl in gill of *Mytilus edulis* showing disorganised nucleus and a distinct sac. × 300. After Herdman and Hornell.

The larval forms which occur in the wall of the gut of the oyster are apparently of the same species as the *Tetrarhynchid* parasites found by the writer in *Balistes spp.* and *Lethinus spp.*, etc. The latter species of fish, and possibly other species of Teleosts, are probably as the writer pointed out in 1910, co-lateral larval hosts.

The globular cystic larva found in the pearl oyster is normally enclosed in a fibrous cyst and, as Herdman showed, it is clear that in this condition it cannot become the nucleus of a pearl. Pearl formation must commence before the fibrous capsule develops, and

there is obviously no reason why this should not occur. Several pearls enclosed in typical pearl-sacs were on many occasions sectioned and found to contain the remains of the larva normally occurring in globular cysts. Jameson (1912) points out that in *Mytilus* the worm nuclei found in the centre of the mussel-pearls are quite large (0·5 mm.) and easily diagnosed. In the Ceylon pearl oyster the pearl nuclei would naturally be very small, if pearl formation took place round larvae at a stage so young that the fibrous capsule had not developed round the larvae. It should be remembered, however, that the essential condition for pearl formation is an epithelial sac, not a nucleus.

Various investigators (Willey, Hornell, Southwell) found that the globular plerocercoid larvae were capable of multiplying endogenously and in this way the infection of the pearl oyster is increased.

Although oysters are, as a rule, heavily infected with these globular larvae, cyst pearls are comparatively scarce. The explanation offered, and it seems a reasonable one, is that it is only the dead larvae which set up irritation. Jameson maintains that the irritation is not mechanical but toxic.

In 1910 the writer, with a view to deciding definitely by experiment what the adult forms of the larvae found in the pearl oyster were, arranged to feed large rays with oysters and observe the result. An area of sixty-four square yards in the open sea at a depth of two fathoms was isolated by a network of expanded metal having a four-inch mesh. A few days later 36,000 oysters were placed in the bottom of the enclosed area. A large ray (*Taeniura melanospilos*) measuring 7 feet 6 inches, and a 'shark' (*Ginglymostema concolor*) measuring 6 feet 6 inches, were captured alive, and, after being treated with male fern extract and castor oil (in order to clear the gut of any parasites they might then have) were placed in the enclosure and allowed to feed on the oysters for twenty-eight days. At the end of that time they were killed and the intestines were found to contain the following parasites :—

G. concolor :	*Tet. unionifactor*, 51.
	Tet. herdmani, 48 (some in stomach).
T. melanospilos :	*Tet. unionifactor*, 150 (in stomach only).

The presence of *Tet. unionifactor* in a shark called for comment, as sharks were not previously known to feed on oysters.

The following year the experiment was repeated. Twelve thousand oysters were placed in the enclosure along with the following fish, which were first treated with male fern extract :—

1 *Taeniura melanospilos*, 4 feet 6 inches.
3 *Rhynchobatis djeddensis*, 5 feet.
1 *Ginglymostoma concolor*, 8 feet 7 inches.
2 *Trygon walga*, 6 feet 6 inches and 3 feet 7 inches.

The specimen of *T. melanospilos* died from the shock of transport and at the end of the third day all three specimens of *R. djeddensis* died.

There thus remained one *G. concolor* and two *T. walga*. As a check experiment two other rays (*Trygon spp.*) were trawled at the same place and at the same time ; one was killed and examined immediately and found to contain numerous cestodes in its spiral valve. The other was treated with 30 minims of male fern extract and killed after three days. Only a very few cestodes were found, but the numerous reddish indentations in the spiral valve clearly indicated the positions of those cestodes which had been dislodged.

Of the fish in the enclosure, one was killed after twenty-four days, and it was found that the parasites had not developed. The rest were killed after having been in the enclosure for forty-seven days.

The following list shows the Cestodes found in their intestines :—

T. walga : Small cystic forms only (undetermined).
G. concolor : *Tet. unionifactor*, 38.
Phyllobothroides hutsoni, n.sp., 140.
Phyllobothroides kerkhami, 9.

The writer does not claim that the experiments were in any sense conclusive. Cestode cysts occur in a large number of marine forms (jelly-fish, various small Teleosts, etc.), and it is quite likely that such forms gained entrance to the enclosure through the mesh. Whether they were eaten or not is open to doubt. The outstanding fact was that although *Tet. unionifactor* had never before been found by the writer in any sharks or rays trawled on the Pearl Banks (and large numbers had been examined over a period of four years), *Tet. unionifactor* was found on both occasions, in the fishes which had been kept in the enclosure and fed on pearl oysters. Further, it was remarkable that no specimen of the genus *Tylocephalum* was

found. That the fish had fed on oysters was evidenced by the fact that the sand at the bottom of the enclosure showed numerous fragmented oyster shells.

Jameson (1912) reviewed the work which had been done on the pearl-inducing worm in the Ceylon Pearl Oyster, and came to the conclusion that the globular larval cestodes found in the pearl oyster belonged to the genus *Tylocephalum*, and not to the genus *Tetrarhynchus*. As the larval forms of *Tylocephalum* occurring in the oyster are of two sizes, he named the larger form *Tylo. ludificans* and he associated this larva with an adult worm found by Hornell in the intestine of *Aetobatis narinari*. As pointed out by Herdman in a note at the end of this paper, the correct name of this parasite is *Tylocephalum unionifactor* (Shipley and Hornell, 1904). It appears to the present writer that *Tylocephalum ludificans*, Jameson, 1912, is identical with *Tylocephalum dierama*, Shipley and Hornell, 1906.

About six species of the genus *Tylocephalum* are now known, and although there can be little doubt that the larva is referable to the genus *Tylocephalum* it is absolutely impossible to say to which species the larva belongs.

The smaller larva Jameson named *Tylo. minus ;* no reference was made to the adult of this form which may, or may not, be synonymous with *Tylocephalum unionifactor.*

Jameson further remarked :

> ' I think there is very good reason to believe that Southwell did, in his feeding experiments actually transmit *Tetrarhynchus unionifactor* from the oyster to the *Elasmobranch*, but it is difficult to escape the conclusion that the worms found in *Ginglymostoma* were derived from the *Tetrarhynchus* larva found in or around the alimentary canal of the oysters and not from the globular *Tylocephali* (*sic*) in the other tissues . . . .'

Although it is undoubted that the globular larvae in the pearl oyster are *Tylocephala* and also that various species of *Tylocephala* have been found in rays (*Trygon spp.*), the most interesting question is why representatives of the genus *Tylocephalum* were entirely absent in the fish fed experimentally. The conclusion one is tempted to draw is that the specimens of *Tetrarhynchus unionifactor* were not developed from the globular cysts, but from the larval *Tetrarhynchids* found in the oyster.

Herdman (Report, Pt. V, p. 21) gives a series of figures showing the

hypothetical way in which a larva apparently belonging to the genus *Tylocephalum* might be transformed into a *Tetrarhynchid*. It appears to the writer improbable that such a transition takes place.

The entire absence of any species of *Tylocephalum* is absolutely unexplained unless one assumes that the globular larvae do actually develop into *Tetrarhynchids*, and this seems improbable.

Jameson, after examining 356 pearls derived with few exceptions from *Margaritifera vulgaris* chiefly from Ceylon, summarised his conclusions as follows :—

'(1) The evidence that the globular Cestode larvae, which Professor Herdman regards as the cause of the formation of "fine pearls" in the Ceylon Pearl Oyster, are a young stage of the worm described by Shipley and Hornell as *Tetrarhynchus unionifactor* is quite inconclusive. I consider these worms to be more probably referable to the genus *Tylocephalum* (or an allied form), and have, provisionally, described them under the name of *Tylocephalum ludificans* and *T. minus*, spp. nn.

'(2) The theory that these Tapeworms are the cause or a cause of the formation of pearls in the Ceylon Pearl-Oyster . . . . is supported by quite insufficient evidence, and even their occasional occurrence in the nuclei of Ceylon pearls has yet to be demonstrated . . . .

'. . . . It is, of course, possible that in certain of the Ceylon banks, conditions may exist which cause *Tylocephalum ludificans* to depart from its normal habit, and acquire an ectodermal instead of a fibrous cyst ; or it might even be found that in certain banks *another* species of *Tylocephalum* (or other cestode) occurs which, like the Trematode in *Mytilus*, normally and habitually gives rise to a pearl-sac in the tissues, and which has been confused with *Tylocephalum ludificans* . . . .

'(3) . . . .

'(4) The " Calcospherules," which Herdman identifies as the nuclei of muscle-pearls, are not free concretions, but are minute pearls formed of the hypostracum or muscle-attachment substance. They are, therefore, not the *cause* of the nacreous muscle-pearls, but a phase parallel to them. There is some reason to believe that the origin of muscle-pearls is associated with pathological invaginations or immigrations of the epidermis at the points where the muscle-attachment epithelium passes over into the ordinary outer mantle-epithelium.

'(5) Parenchyma-pearls (which name I apply to Professor Herdman's cyst-pearls) may be formed around grains of sand or other foreign particles, organic granular matter of doubtful origin, or bodies composed of varieties of the shell-substance which arise when the normal rhythm of secretion is disturbed (repair-substance). A foreign nucleus is probably rather exceptional. The ultimate factors which give rise to the epidermal sacs in which they are formed have yet to be discovered. Many of them are probably of the same origin as muscle-pearls, except that they arise singly at points where a few muscle-fibres are inserted into the shell, instead of in clusters at the regular muscle-insertions. The dark pseudo-nuclei of these pearls, which may easily be mistaken for the remains of the parasites, are usually composed of the repair-substances.'

Seurat, working on the parasites of the black-lipped Pearl Oyster (*Margaritifera margaritifera* var. *cumingii*, Reeve) of the Gambier

Archipelago, came to the conclusion that pearl formation was due in that species of oyster to a parasite which Giard (1903) placed near the genus *Cyathophyllus*, Kessl (= *Acrobothrium*, Olsson = *Tylocephalum*, Linton).

Seurat, later (1906), correlated this larva with an adult worm found in the intestine of *Aetobatis narinari*, which he named *Tylocephalum narinari*. There is, however, nothing to show that the young form belongs to that particular adult. The point of interest in these observations is the fact that Seurat believed that the globular larva was connected with pearl formation in *M. margaritifera* var. *cumingii*, Reeve.

Hornell (1922) summarises his later opinions on the question as follows :—

'The origin of these pearls has been a battlefield of theory in the past ; the resultant confusion appears to me to be due in large part to the lack of recognition that there are these two main categories of pearls, differing in origin, and that in the case of cyst-pearls the causative body may, and usually does, differ with the locality and the species investigated. In the case of certain mussels (*Mytilus edulis*) the causative nucleus has been found in certain beds in France, to be a larval trematode worm (Jameson and Boutan), and in certain fresh-water mussels in one locality this is replaced by a little commensal mite (Küchenmeister). In the case of the Ceylon and Indian pearl oyster, Professor Herdman and the author found it in many cases to consist of the dead body of a larval Cestode. To this we gave the name *Tetrarhynchus unionifactor*, and we correlated it with an advanced larval Tetrarhynchid of typical form found, commonly, encysted in the walls of the oyster's intestine. At a later date we discovered that the adult of the latter worm is found in the sexually mature condition in the intestine of an oyster-eating ray, *Rhinoptera javanica*. At one time we intercalated an intermediate host, one of the file-fishes (*Balistidae*) but, eventually, the species found in the file-fishes was found to be of a distinct species, not parasitic in the larval condition in pearl oysters. I have, however, come now to the conclusion that the spherical cestode larva found in abundance in the tissues of the pearl oyster and frequently as a nucleus in cyst pearls from the same mollusc, is not a younger stage of the undoubted *Tetrarhynchid* larvae encysted in its intestine. Possibly it is the larva of some species of *Tylocephalum* or other closely related genus, but this is a subject for further investigations.

'Few pearl oysters are free from this parasite. Usually the gills contain hundreds, often very minute . . . . The digestive gland is another favourite location for these cysts, opalescent white spheres conspicuous in the dark green of the gland.'

Hornell showed

'Two nuclei which I obtained by decalcification of small orient pearls ; there can be no question as to their identity with the spherical larvae found alive in the tissues. Neither Professor Herdman nor I ever claimed that all cyst pearls have such nuclei ; we recognised that other foreign bodies, notably grains of sand, occasionally function as the intrusive irritating factor and become pearl nuclei. We have also even found a small nematode worm, coiled upon itself, forming the nucleus. So far we went sixteen years ago. Subsequent investigation

shows me that a further qualification is necessary whereby cyst pearls may be divided into two sections, the one comprising pearls induced by the irritation of foreign bodies and the other those with nuclei of periostracal-like substance derived from the oyster's own tissues. The former class comprises, according to my investigations, the majority of the larger cyst pearls, the latter of the smaller ones of this description, which, as I have indicated above, constitute by far the larger proportion of cyst pearls. This conclusion of our local researches disposes satisfactorily of certain objections levelled at the cestode theory, and places the latter in its proper perspective ; we see that cestode larvae, though less frequently the cause of pearl formation than was at first believed, are nevertheless the *most important factor in the production of the larger and finer of Orient pearls* and, therefore, of supreme importance from the economic and commercial view-point. Let us now see how pearl formation proceeds in cyst pearls formed around intrusive foreign bodies (*b*) . . . . (*c*) . . . .

'Some of my earliest experiments made in Galle in 1902, have direct and fundamental bearing on this problem. These were in respect of the power of the oyster to repair injuries to the shell. They resulted in demonstrating that epithelial cells are capable, at least over the nacre-secreting area, of an alteration in the character of their secretive power upon emergency. Thus I found that if a fragment of shell in the centre of the valve were removed, exposing the mantle which, previously, had been engaged in secreting nacre, the first repair substance formed was not nacre, but a yellow parchment-like material apparently identical with periostracum. Only after a stiff layer of this was formed, was there a resumption of nacre secretion. Now in all the pearls I have examined and, notably, in button pearls formed after the old Chinese method, and within recent years refined and extensively employed on a commercial scale by the Japanese, I have found that the nucleus, whether it be a cestode larva, a grain of sand, or a spherule of mother-of-pearl (as in the Japanese culture pearls), is not over-laid directly by a nacreous layer, but has interposed between its surface and the eventual layers of nacre, a distinct and well-marked deposit of stiff yellow membrane identical with repair periostracum, which, indeed, it is. It is evident that the intrusion of any body into the ectoderm must affect it in a similar manner to that caused by a direct injury, such as a fracture of the adjacent shell would do; hence the impulse of the cells around the intrusive body is to pour out the primary secretion employed to meet such an eventuality. The inmost layer of such a pearl is invariably of periostracum. Only after the effects of the shock have passed and normal conditions are restored, does the nacre secretion begin to be again deposited. What seems to me to be the explanation is that the membrane repair substance is really the conchyolin basis of nacre with the lime salts withheld. In other words, after a shock, the epithelial cells intermit the secretion of lime salts, but continue the secretion of conchyolin, thus giving a periostracal appearance to what would normally be a nacreous layer (conchyolin + carbonate of lime).

'Another deduction which I have made from the investigation, is that only dead or dying parasites excite irritation of the character necessary to induce pearl formation. A living parasite does not irritate the tissues in the same way ; indeed, it merely induces the formation of a tough connective tissue sheath or cyst enveloping it wherein it lies quiescent and harmless, giving no further irritation. But in the case of a parasitic larva that arrives in the epithelium in a dying condition, exhausted or perhaps smothered in the secreted fluid poured out by the epithelial cells, a different situation is found. Instead of being within a layer of connective tissue, it lies in a depression of the epithelial layer of cells and these act differently from connective tissue cells—with a correspondingly divergent result.'

In a private letter to the author, Hornell states that his

'Latest opinion is that the pearl larva which was first put down as a larva of a *Tetrarhynchid* Cestode and named in consequence *Tetrarhynchus unionifactor*, is not the larva of a *Tetrarhynchid* at all, but is the larva of a Cestode of some other genus—which is more likely to be *Tylocephalum* than any other. But I consider that the adult of this larva is not as yet identified.'

SUMMARY

We may now summarise our present knowledge with reference to the so-called pearl-inducing worm in the Ceylon Pearl Oyster as follows :—

(1) Herdman and Hornell (1902 to 1906) found a number of globular cestode larvae in the tissues of the pearl oyster which they concluded were the principal causative agent in pearl formation. The larva was actually found to be the nuclei of several pearls examined by Herdman and Hornell. This larva (the adult form of which occurs in various Elasmobranch fishes) was named *Tetrarhynchus unionifactor* by Shipley and Hornell in 1904. Herdman gave hypothetical diagrams showing the manner in which he considered the globular larvae might become transformed into *Tetrarhynchids*.

(2) At least three different kinds of Cestode larvae inhabit the tissues of the oyster, viz. (i) a larval *Tetrarhynchid* in the intestines of the pearl oyster and (ii) two different sizes of globular larvae found in various parts of the tissues of the oyster, and belonging apparently to the genus *Tylocephalum*.

(3) Seurat (1906) concluded that the causative agent in pearl formation in the pearl oyster of the Gambier Archipelago was a Cestode larva belonging to the genus *Tylocephalum*.

(4) Southwell (1910 and 1911), as a result of feeding experiments, obtained *Tetrarhynchus unionifactor* (and other Cestodes) but no representative of the genus *Tylocephalum*, and concludes that the specimens of *Tet. unionifactor* were obtained from the larval form of *Tetrarhynchus* found in the oyster's intestine and not from the globular cysts. Why the adult of the larvae in the globular cysts was not obtained is not understood, and remains a matter of some significance.

(5) Jameson (1912) states that the globular larvae in the pearl oysters represent two different species of *Tylocephalum* and that the

theory that tapeworms are the cause or a cause of pearl formation in the Ceylon Pearl Oyster is supported by quite insufficient evidence ; he points out that the larval cestode always occurs in a fibrous sac, whereas an epidermal sac is necessary before a pearl can be formed and also that parasitic infection apparently bears little relationship to pearl formation.

It should here be noted, however, that pearl formation was only presumed to take place round larvae which for some reason or other had died very early on, and as a result set up local irritation.

Jameson sectioned a considerable number of pearls and was unable to find any trace of a Cestode parasite in the centre. He further concluded that the nucleus of Ceylon pearls consists of grains of sand or other foreign particles or organic matter of doubtful origin, or bodies composed of varieties of shell-substance which arise when, through any cause, secretion is disturbed.

(6) Hornell (1922) agrees that the globular cysts in the pearl oyster belong to the genus *Tylocephalum*, and states that *Tetrarhynchus unionifactor* is to be correlated with the advanced larval *Tetrarhynchid* commonly found encysted in the wall of the oyster's gut.

The difficulty of arriving at a definite conclusion in the matter will be evident from the foregoing, but the following points appear to be well-established :—

(1) That the globular larvae in the pearl oyster belong morphologically to the genus *Tylocephalum*, and probably *Tetrarhynchus unionifactor* is the adult of the *Tetrarhynchid* larva occuring in the walls of the gut of the oyster.

(2) The reason why no representative of the genus *Tylocephalum* occurred in the fishes which had been specially fed on oysters is unknown. Experimentally, the globular larvae appeared to develop into *Tetrarhynchids*. It is desirable that feeding experiments should be tried again, on a bigger scale, and for a greater length of time, in order to decide definitely whether the larva does belong to the genus *Tylocephalum* or whether, as Herdman suggested, the globular larvae actually develop into *Tetrarhynchids*.

(3) There is no doubt that the globular larvae do frequently occur as pearl nuclei and that the pearl formation round them only takes place when, for any reason, a young larva dies before a fibrous cyst is formed and sets up local irritation. The fact that a

pearl sac of epithelial origin occurs round such pearls is established beyond doubt.

(4) Whilst it appears to be true that these dead globular larvae are the primary cause of pearl formation it is probable that other bodies form the nuclei of pearls, such, for instance, as grains of sand, amorphous shell substance, dead organic particles, etc.

(5) It would appear that from the financial or commercial point of view the value of these fisheries depends not entirely on their regularity and magnitude, but also on the number of pearls contained in the oysters. It is not unreasonable to assume that the yield of pearls could be increased if numbers of the globular larvae in the oyster could be killed by artificial means, whilst the oyster was alive and young. After such treatment the oysters could be returned to a localised area in the sea and left to grow. The writer is well aware of the fact that operations of this kind would be difficult, but they are certainly not impossible.

NOTE added by Sir William Herdman, January, 1924.

After consideration of the further investigations that have been carried out by Southwell, Hornell, Seurat, and others, during the last twenty years, I am now inclined to think that the globular cysts in the liver of the Ceylon Pearl Oysters which Hornell and I found in 1902 and regarded as larval stages of a *Tetrarhynchus* and which were formally described by Shipley and Hornell in 1904 under the name *Tetrarhynchus unionifactor*, are—as Mr. Southwell says in the present paper—more probably to be referred to the genus *Tylocephalum*. If that is so, by the rules of zoological nomenclature, the correct name of the parasite comes to be *Tylocephalum unionifactor* (Shipley and Hornell, 1904) in place of *Tylocephalum ludificans* suggested by Jameson in 1912.

Southwell's experiments at Ceylon in feeding fishes on pearl oysters are most important and should be repeated in order to test further the curious result that although the '*Tylocephalum*' cysts are most abundant in the oysters, the resulting parasites in the fish are chiefly *Tetrarhynchids*.

W. A. H.

Since the above paper was written, Professor Sir William Herdman has drawn the writer's attention to a paper in which Dollfus (1923) records the occurrence of Cestode larvae belonging to the genus *Tylocephalum* as nuclei of pearls in *Meleagrina occa*, Reeve, and *M. irradians*, Reeve. Dollfus states that the adult of the parasite is not known, but that the larva does not appear to differ from the globular larvae in the Ceylon Pearl oyster. These, undoubtedly, belong to the genus *Tylocephalum*. Dollfus considers that the *Tetrarhynchid* larva in the Ceylon Pearl oyster is quite different from the globular cyst which occurs in the same mollusc.

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THE RELATIVE NUMBER OF MALE AND FEMALE CRESCENTS

BY

J. W. W. STEPHENS

AND

R. M. GORDON

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Most of the standard text-books contain little, if any, information on this subject, or at least what there is, is confined to some general statement unsupported by satisfactory data. A search through the literature, not an exhaustive one we admit, has furnished us only with two records.

Knowles (1919) gives the following table :—

TABLE I.

Date	Total number of crescents counted	Macrogametocytes	Microgametocytes
25.11.17	27	23	4
27.11.17	56	49	7
28.11.17	29	25	4
29.11.17	19	18	1
30.11.17	45	41	4
1.12.17	31	29	2
2.12.17	26	25	1
5.12.17	3	3	0
7.12.17	16	15	1
11.12.17	14	14	0
12.12.17	4	4	0
13.12.17	9	8	1

Although the number of crescents counted by Knowles is small, yet on all occasions he found the females in excess, and if we take the average of his figures we get the values, females 21, males 2, i.e., the females are ten times as numerous as the males.

We have compiled the following table from the data given by Abrami and Senevet (1920), but have omitted the figures they give showing the corrections for the statistical error, as the number of crescents counted was sufficient in most cases to exclude any large error due to this cause.

TABLE II.

Section I.

Date.	Total number of crescents counted	Females %	Males %
25.10.20	1500	52	44
26.10.20	1500	47	51
27.10.20	1500	41	53
28.10.20	1000	52	44
31.10.20	400	45	47
1.11.20	200	56	38*
2.11.20	83	55	38
3.11.20	85	62	30

Section II.

	175	59	29
	1015	56	41
	400	71	23
	200	76	19

* The author's figure is 27·7, probably a misprint for 37·7.

The first section of the table refers to the same case counted on various days, and the second section to four separate cases. It will be noted that the percentages do not add up to exactly 100, as the authors found that in certain cases they were unable to decide whether a crescent was a male or a female.

If we now take the average of the figures in the first section we get the following values: Females 51, males 43 (1·2 to 1).

It is noteworthy that on three occasions the males were in excess.

In the second section of the table we find that the figures for the females and males vary considerably, the females, however, being always in excess. If we take the average, the figures are, females 65, males 28 (2·3 to 1).

The counts made by ourselves were on a series of thin blood films sent to one of us by Dr. E. A. C. Smith, Federated Malay States. It may be of interest to state that they were made from the blood of a case of general paralysis of the insane, inoculated with simple tertian malaria (*P. vivax*) on 11 July, 1923. Simple tertian parasites appeared in the blood on 25 July, 1923, and crescents on 4 August, 1923 (the source of the *P. falciparum* infection is obscure), and in nearly all the films were so abundant that 1,000 crescents could be easily counted. Our experience has been similar to that of Abrami and Senevet, that occasionally we were unable to decide whether a crescent was a male or a female. If the films were deeply stained there was a tendency to call females male, and in such films care was required in distinguishing them, but in less deeply stained films the distinction was well-marked.

The figure for these doubtful elements, which was certainly less than 5 per cent., we have omitted from our counts.

Five hundred crescents were counted in each film by each of us, the one not knowing previously the figure obtained by the other. The difference in individual counts ranged from 4 to 15 per cent.; the average being about 10 per cent. This may be due to the fact that the proportion of females to males varied in different parts of the film, or may be due to a difference in interpretation.

The results obtained by us are given in Table III.

Table III.

Date	Total number of crescents counted	Observer A. Percentage of		Observer B. Percentage of	
		Females	Males	Females	Males
5.8.23	500 + 500	72	28	76	24
6.8.23*	500 + 500	70	30	85	15
8 a.m.					
6.8.23*	500 + 500	73	27	88	12
3 p.m.	500	75	25		
7.8.23*	500 + 500	72	28	76	24
3 p.m.					
8.8.23*	500 + 500	67	33	78	22
3 p.m.					
9.8.23*	500 + 500	66	34	76	24
3 p.m.	500	68	32		
10.8.23*	500 + 500	68	32	78	22
3 p.m.					
12.8.23*	500 + 500	72	28	82	18
3 p.m.					
14.8.23*	500 + 500	66	34	74	26
8 a.m.					
Average		70	30	80	20

* Quinine hydrochloride grains 20.

From Table III we get the following average figures: for A, females 70, males 30 (2·3 to 1); for B, females 80, males 20 (4 to 1); and for A and B combined, females 75, males 25 (3 to 1).

Finally we summarize the observations in Table IV.

TABLE IV.

Authority	Case	Total number of crescents counted	Average ratio $\frac{\text{Females}}{\text{Males}}$
Knowles (1919)	Blood films made from 1 case on 12 different days.	279	$\frac{10}{1}$
Abrami and Senevet (1920)	(1) Blood films made from 1 case on 8 different days.	6268	$\frac{1.2}{1}$
	(2) One blood film from each of 4 cases.	1790	$\frac{2.3}{1}$
Stephens and Gordon (1924)	Blood films from 1 case on 9 days	10000	$\frac{3}{1}$

CONCLUSION

In a case observed by us on nine separate days, female always outnumbered male crescents, the average ratio being three to one.

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ADDENDUM

In a blood film made on 15.11.23 by Dr. Govadham, Central Provinces, India, and received by us on 31.3.24, 1,000 crescents were counted. The ratio of females to males was 750 to 250, i.e., 3 to 1.

TROPICAL EAR

BY

The late J. M. FORSYTH, M.B., Ch.B. (Glasgow).

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PLATE IV

This condition is known in the Malay states as ' Singapore Ear,' but it is prevalent in all tropical or semi-tropical climates. In Calcutta it is called ' Calcutta Ear,' in Java ' Java Ear,' in Hongkong ' Hongkong Ear,' but the comprehensive term ' Tropical Ear ' is, perhaps, more suitable.

So far as I can find out no definite attempt has been made to investigate the nature of this condition, notwithstanding the fact that it is very common among all classes, European as well as native.

During the past twelve months I have examined and made cultures from over a hundred cases.

' Tropical Ear ' may be defined as :—A localised infection of the external auditory meatus which does not involve the middle ear, but sometimes, though rarely extends into it.

From the etiological findings it is possible to divide it into two main groups, viz. :—

I. Otomycotic.
II. Bacterial.

Group I. OTOMYCOTIC

Isolated cases of fungus in the ear have been found by many investigators. Thus Castellani and Chalmers give the following :—

Mucor pucillus, Lindt, 1886 ; *Lichtheimia corymbifera*, Cohn, 1884 ; *Lichtheimea ramosa*, Lindt, 1886 ; *Saccharomyces ellipsoides*, Rhees, 1870 ; *Monilia rhoi*, Castellani, 1909 ; *Aspergillus flavus*, De Bary, 1840 ; *Aspergillus flavus*, De Bary, 1870 ; *Aspergillus malignus*, Lindt, 1889 ; *Aspergillus repens*, De Bary, 1870.

In the fifteen cases met with, fourteen were found to be due to an *Aspergillus*, and one was found to be a yeast. As the yeast infection differs so much from the others and is an isolated case,

it has been described under ' Accidental Infections ' in the bacterial group, though it belongs to the *Saccharomyces*.

Among the fourteen cases of Aspergillus Infection, four distinct colour varieties were found :—

(1) Black. (5).
(2) Reddish brown. (1.)
(3) Mouse-coloured. (1.)
(4) Green in three shades ; Blue-green, Olive-green, Grass-green. (7.)

Aspergillus niger is larger than the other species, is more virulent, and grows more quickly.

Green aspergillus differs from *A. niger* in the following points :—

Colour : (*a*) Blue-green ; (*b*) Olive-green ; (*c*) Grass-green. It has a more delicate structure. It has finer sterigmata. It is smaller. The diameter of the spores is from 3-4 microns. The growth soon withers and the colour fades, though the spores remain alive for long periods.

The different varieties, Blue-, Olive-, and Grass-green breed true.

Brown aspergillus culture has a reddish brown colour and spores 3 microns in diameter. Only one case seen. Breeds true on sub-culture. Otherwise has the characters of an *Aspergillus*.

Mouse-coloured aspergillus, except for the colour, does not differ from the brown variety. Breeds true on sub-culture.

Clinical Manifestations. The symptoms were the same in all fourteen cases, though the duration and severity varied slightly.

In the early stage itching and irritation of the meatus are the only signs ; later, deafness and a feeling of ' fulness ' in the ear become more and more marked ; later still the irritation increases to actual pain. On examination of the meatus the picture is fairly characteristic. If wax is present, the only indication is the presence of white caseous looking material, mixed with the normal brown wax. If wax is absent, the white furry growth can be clearly seen on the walls of the meatus, and sometimes on the drum itself. Later the canal becomes completely choked with this white caseous material and the brown wax disappears, showing that the fungus grows on the wax and absorbs it. If left undisturbed, this white caseous material becomes crinkled and crenated and looks very

like a mass of sodden desquamated epithelium, for which it is often mistaken.

Clinically, except for slight redness of the walls of the meatus and occasionally slight opacity of the drum, no evidence of disease can be detected, once the meatus has been thoroughly syringed and swabbed out. There is no tenderness of the auricle or auricular glands; no fever or malaise; and the slight amount of deafness is due to the mechanical blocking of the ear.

TREATMENT. As many cases do not come for treatment until the condition is far advanced, some difficulty may be met in clearing the meatus, which is choked with a thick growth of fungus. It may be necessary to pick it out piece-meal with forceps. But in the majority of cases, softening with pure hydrogen peroxide and syringing with hot water and peroxide will dislodge the main bulk of the growth. The meatus and drum can then be swabbed clean of the remaining portions, which sometimes cling tenaciously, especially in the anterior recess between the drum and meatal wall; a strong antiseptic should be used in doing this, such as S.V.R. (rectified spirit of wine).

To all appearances the meatus is free from growth and the patient cured; but a recurrence is likely unless a more systematic treatment is adopted.

The following routine is suggested :—

First day—

(1) Instil pure peroxide into meatus and allow to act for two minutes.

(2) Syringe with hot water and peroxide until bulk of growth is dislodged.

(3) Swab out with dry wool, paying particular attention to the anterior recess and the surface of the drum.

(4) Swab out with hydrarg. perchlor. 1 gr. to the oz. of S.V.R.

(5) Pack meatus with wool soaked in a solution of perchloride in spirit $\frac{1}{4}$ gr. to the oz.

Second and Third days.

Repeat above.

Fourth day.

Clean as before, instil drops of perchloride in spirit (1 gr. to the oz.) and pack with dry wool.

The patient should carry on the daily treatment himself for one week as follows :—

(1) Instil pure peroxide and allow to act for two minutes.

(2) Dry thoroughly and instil two drops of perchloride in spirit ($\frac{1}{2}$ gr. to the oz.).

(3) Pack with dry wool.

Weekly Inspection should be made for a month and treatment repeated as on the first day. As well as this, the patient should on two days in each week, instil drops of carbolic in glycerine (1 in 5). In persistent cases it is advisable to vary the antiseptic and the following have been found effective :—

(1) Proflavine in saline 1-1000, or 1-500. The ear can be packed with wool soaked in this. The only objection to its use is that it gives rise to great itching, for some days after.

(2) *Linimentum belladonnae* and *Linimentum opii* equal parts. This can be painted over the walls of the meatus ; or a pledget of wool soaked in it can be packed into the ear. The burning pain to which it gives rise, passes away in about half-an-hour.

(3) Zinc sulphate gr. 10, cocaine hyd. gr. 2, boric acid gr. 10, S.V.R. $\frac{1}{2}$ oz., aqua ad. 1 oz. This can be used as ear drops twice daily, three days in each week for a month.

Prophylactic Treatment.

(1) Keep the ears as dry as possible.

(2) Cleanse the ear daily with 3 per cent. mercurial soap.

(3) Pack the ears when bathing with wool and sterile vaseline.

The following illustrative cases are of interest :—

Case 1. A. J. 1st September, 1920. Complains of fulness and discomfort in the ear. Meatus completely blocked with thick white plug. Removed with forceps and syringing, when large perforation is seen in anterior sup. quadrant.

21st September. Meatus almost choked again. Black mould growing on walls, flowers and stalks clearly visible. Perforation unchanged. Complains of discomfort but no pain.

25th September. Mould still present. Flowers clearly seen. No symptoms.
29th September. Mould still present.
30th September. No mould visible. Perforation unchanged.
1st October. No mould present.
3rd October. Mould again visible.
5th October. No mould. Perforation smaller.
7th October. No mould. No symptoms. Perforation smaller but permanent.

The *treatment* adopted was that given above.

Case 2. A. H. 13th October, 1920. Both ears blocked with white macerated material. Complains of great discomfort and deafness.

25th October. Ears clear.

28th October. Fungus again present. No symptoms.

22nd November. No symptoms, but small amount of white fur in depths of meatus from which *Aspergillus niger* grew.

7th March, 1921. Small amount of fungus in both ears. Complained of slight irritation for two days previously.

The above case was one of a traveller who could only come for a day or two for treatment, with long intervals in between.

The *treatment* was on the lines already indicated.

Case 3. E. S. M. 18th April, 1921. Deafness and great pain in ear of one day's duration; history of discomfort and increasing deafness for past ten days. Meatus completely blocked with white caseous material. This was so tough that it had to be picked out with forceps before the meatus could be syringed out. Portions of desquamated epithelium came with it, leaving a raw red surface on the walls and an acutely inflamed drum. A minute perforation of the drum present. (Culture made).

Carbolic and glycerine (1-5) drops packed in, as inflammation too severe to apply spirit.

19th April. Meatus clear. Drum greatly inflamed, with white patch of fungus on it surrounding the perforation which is distinctly larger. (Culture shows *A. niger*).

White patch on drum carefully removed and whole ear swabbed out with perchloride in spirit. The perforation healed rapidly.

Group II. BACTERIAL

From the etiological findings it is possible to divide this group into three sub-divisions:—

(1) Simple.
(2) Accidental.
(3) True Tropical Ear.

SIMPLE INFECTIONS

Symptoms.

Irritation in the ear, feeling of fulness, slight deafness. As a rule the meatus is blocked with wax; or there may be a slight mucopurulent discharge, together with a sodden appearance of the walls of the meatus.

Etiology.

The culture media show the presence of a mixed infection, comprising *Staphylococcus albus*, and *S. aureus*, *Micrococcus catarrhalis*, large diplococci, *Bacillus coli*, occasionally a few streptococci. In one case *S. aureus* in pure culture was found.

TREATMENT.

This condition readily clears up under treatment. Peroxide drops, followed by syringing with warm antiseptic lotion, drying thoroughly, and finally drops of perchloride in spirit, the ear being packed with dry wool; one thorough cleaning as above is often sufficient. The patient should, nevertheless, use glycerine and carbolic drops, for three days.

ACCIDENTAL INFECTIONS

In this group are included those rare infections that could not be grouped elsewhere. The following case was due to a *Saccharomyces* infection.

CASE 1. B. 26th October, 1920. Complaining of pain and discharge from the ear and slight deafness. Meatal walls covered with this mucopurulent material. Meatus inflamed; small yellow vesicles seen on walls and drum. Drum very much inflamed with a yellow patchy appearance. No bulging of drum and no appearance of middle ear infection. No history of sore throat. (Culture made).

27th October. Great pain during the night, which passed away gradually, meatus full of glairy muco-pus, pale yellow in colour. Large perforation of drum in anterior quadrant.

30th October. Much cleaner. Drum less inflamed but still shows the peculiar yellow parchy appearance. Perforation slightly smaller. Culture shows a pure growth of a yeast.

1st November. No pus; greatly improved. Yellow patches on drum still present.

3rd November. Improved. Perforation unchanged.

18th November. No pus or inflammation. Ear dry. Perforation much larger, has extended on each side into the yellow patches.

2nd December. Perforation smaller.

18th March. Perforation much smaller, but apparently permanent.

Treatment. The double liniment was used daily for the first week. After that, perchloride in spirit.

Case of mixed *B. diphtheriae* and *B. coli* infection.

CASE 2. E. 18th July, 1921. Complains of pain in the right ear and slight deafness. Meatus full of yellow pus slightly glairy in consistence, walls slightly inflamed, drum inflamed but intact, no evidence of middle ear infection. Anterior recess filled with fungus-like material.

27th July. Discharge copious; thin, glairy, pale yellow in colour. Meatus painful and swollen. The symptoms had been quiescent and only reappeared the previous day. (Culture made).

29th July. Discharge copious of a thin, glairy, colourless appearance. Pain gone, swelling less. (Culture shows presence of *B. coli* and a *Diphtheroid bacillus*). (Sub-culture made).

30th July. Discharge very copious. Pain gone; swelling less. (Sub-culture shows *B. diphtheriae*).

(Second sub-culture made). The second sub-culture showed pure growth of *Diphtheria bacillus.*

31st July. Copious, translucent, glairy fluid. Slight swelling but no pain.

1st August. Unchanged.

2nd August. Complained of feeling drowsy the previous day. Discharge less, swelling of walls of meatus almost gone. White membrane seen on drum and adjacent wall, filling anterior recess. (Previously obscured by swelling of walls). Membrane very adherent.

3rd August. Discharge less. Some of membrane removed by careful swabbing, but too broken up for examination. Improvement continued and on 6th August no sign of inflammation was left except a white mark on drum.

28th August. Recurrence of all symptoms which cleared up after a week's treatment, and did not again return.

Treatment. First day. Cleaned and packed with carbolised glycerine. Drops of glycerine and carbolic given for daily use. When the Diphtheria Bacillus was found, the ear was packed daily with perchloride in spirit (1 gr. to the oz.) and 4,000 units of antitoxin were given subcutaneously into the abdominal wall. This treatment made no impression on the quantity of the discharge, but the nature of the discharge changed slightly; the yellow tinge disappeared. The walls of the meatus and the drum were then painted with Proflavine 1 in 100 saline. This also had no effect.

1st August. Pure peroxide two minutes, syringing with warm water and peroxide; drying thoroughly; painting the whole interior with antitoxin and packing ear with wool soaked in antitoxin. Improvement was noticed at once. This was repeated for six days, by which time all signs of infection had completely disappeared. The patient was told to continue with the glycerine and carbolic drops for a day or two. Three weeks later the whole condition reappeared, but completely cleared up after a further course of treatment with antitoxin.

This was an interesting case as it showed a double infection with *B. coli* and *B. diphtheriae.* The *B. coli* disappeared under ordinary antiseptic treatment, while strong antiseptics such as perchloride in spirit (1 gr. to the oz.), pure peroxide, and proflavine 1 in 100, had no effect on the diphtheria bacillus. Antitoxin acted at once and with great effect when applied locally. As no perforation was present the infection did not come from the throat or nasopharynx; and repeated swabs from both these localities showed the absence of *B. diphtheriae,* proving that the infection was a primary infection of the external auditory meatus.

TRUE TROPICAL EAR

The third type of bacterial infection is distinguished, firstly, by a constant group of clinical symptoms and, secondly, by its etiology.

SYMPTOMS. Onset always acute. Malaise marked, fever between

99°-100°. Pain acute; unilateral, though other ear may become infected later.

The pain is centred in the external auditory meatus, but diffuses widely over the same side of the head and may give rise to a severe, throbbing headache. May be agonising in character, excluding all possibility of sleep. Together with the throbbing ache, sharp shooting pains are an alarming feature. Moving the jaw greatly aggravates the pain.

Tenderness a very marked feature, and usually involves the whole of the pinna, the anterior and the inferior auricular glands and sometimes the superior cervical glands. It is centred in the meatus where even the slightest touch is unbearable. No tenderness over mastoid (provided the pinna is not touched). The anterior and inferior auricular glands, and sometimes cervical glands are also involved. Pinna swollen. Walls of external auditory meatus swollen throughout its entire length, and as a rule the canal is obliterated by apposition of the walls. The walls are so swollen that it is often impossible to insert even a fine probe between them. But as acute pain is practically the first symptom, the patient usually comes for treatment before the swelling has obliterated the canal. No swelling over mastoid region.

Presence of small vesicles. Greenish-yellow in appearance on the walls of the meatus, which are thin walled and filled with yellow pus with a faint green tinge in it. When the vesicle is removed, a raw red surface is left. In the later stages it is possible to trace a minute sinus running down from each vesicle towards the cartilage of the pinna, from which pus can be seen exuding. The swelling is at first local, but it rapidly gives rise to intense inflammation, which causes swelling of the whole extent of the canal.

The Drum remains unaffected except for slight inflammation. In no case has perforation resulted.

Course. The condition runs the following course:—Infection of ear; formation of vesicles on walls of meatus; pain and tenderness and swelling commence. Infection quickly spreads into deeper zones; inflammation of the cartilage, which gives rise to intense pain; the swelling of walls of canal, which becomes acutely tender; tenderness of pinna and swelling of glands follow. The next stage is the formation of a deep-seated abscess, which does not 'come

to a head ' and burst, but burrows out a minute channel, which discharges the pus in minute quantities at irregular intervals, always leaving infection behind. Each time pus is evacuated the symptoms moderate and sometimes disappear altogether ; but in a few days, often at the end of a week even, they reappear and the whole condition flares up again. A chronic abscess and the sinus is formed eventually and this may go on to necrosis of the cartilage lining the meatus or forming the pinna, with sometimes sloughing of considerable areas of tissue.

The condition may continue for several months. This is due to the fact that the infection remains deep seated and even spreads along the deeper planes before coming to the surface ; when it does come to the surface it often reinfects another spot on the meatal wall. At no time, throughout the course of the disease, does pus appear in large quantities, as in middle ear infection.

It soon became evident that it was only when *B. pyocyaneus* was present, that symptoms of an intense character followed. And later it was found that in the majority of cases, the cultures showed almost pure growths of *B. pyocyaneus*, and in many cases, no other organisms were found even after prolonged search. The mixed infections usually included mild non-virulent organisms, such as *Micrococcus catarrhalis*, *Bacillus coli*, or large gram-positive diplococci. It was rare to find staphylococci or streptococci, thus differentiating the condition from middle ear infections and furuncle.

In the mixed infections, *B. pyocyaneus* outlived the other organisms, which were soon killed off by the antiseptic treatment. In the chronic cases where the pus came from a deep seated abscess, *Bacillus pyocyaneus* grew on media in almost pure culture. As the meatus is open to the outside air, it is not possible to make a culture which is uncontaminated, and, therefore, the results are all the more striking. In the hundred odd cases referred to, however mild the symptoms were, cultures were made. In many of these the medium remained sterile and no growth resulted, in the majority only slight growth of mild organisms appeared, showing that normally the walls of the meatus are more or less sterile. When, therefore, we find a virulent organism present, and associated with it, a constant set of symptoms not present in other infections, when we again and that in a large proportion of these cases, the infection with

B. pyocyaneus is a pure one, we must come to the conclusion that the germ is the cause of the disease; in other words, that *Bacillus pyocyaneus* is the cause of 'Tropical Ear.'

TREATMENT

Mild Cases include those which come early for treatment, some of which may in spite of treatment pass on into the severe stage.

If the meatus is choked with wax, it is necessary to syringe with warm water and peroxide; but unless it is absolutely necessary to clear the ear, do not syringe. This is an important point, because any moisture in the ear tends to make the walls of the meatus sodden, and a nidus is formed of softened dead epithelium, in which the germs propagate and through which they rapidly penetrate. After the ear has been cleansed and dried thoroughly, examine the walls of the meatus for vesicles; any found should be removed with forceps, and the pus absorbed on dry wool. Then instil a few drops of pure peroxide and allow to act for a few minutes, dry again carefully, and then swab the whole canal with perchloride in spirit. Finally, pack the ear with wool soaked in proflavine in saline 1-1000. A smart saline purge should be given and powders containing aspirin gr. 4, phenacetin gr. 4, caffeine citras gr. 2, taken thrice daily.

The best way to reduce the swelling and pain is to apply a pad of hot antiphlogistine, about half-an-inch thick, all round the ear and over the pinna, and keep it on all night.

Repeat the above treatment on the second day, but pack the ear with perchloride in spirit. If vesicles are present on the third day, swab out the whole canal with *Linimentum belladonnae* and *Linimentum opii*, equal parts. In ordinary mild cases the above treatment will clear up the condition, provided the patient uses peroxide drops followed by perchloride in spirit daily for another week, when the meatus should again be inspected and thoroughly cleansed, and then packed with wool coated with a mixture of *Ung. hydrarg. ammoniatum* and *Ung. acidi borici* in equal parts. If the condition does not clear up in a week, or recurs, it passes into the severe and then into the chronic stage.

Severe Cases always imply the formation of deep-seated abscesses. If the infection can be checked at once, as above, abscesses will not form, but once the germ penetrates into the deeper layers the above treatment will not avail.

If the canal is not completely obliterated by swelling, pack the whole canal with wool soaked in equal parts of *Ung. belladonnae* and *Ung. opii.* This is painful at the time, but the pain passes away in about half-an-hour. Apply a pad of very hot antiphlogistine about half-an-inch thick all over the ear and down into the entrance of the meatus. This should be renewed at the end of six hours, and in the interval heat should be kept applied by means of a hot-water bottle on the pillow. Give a calomel purge. Do not syringe the ear as the point of the syringe in the meatus causes great pain and the syringing serves no useful purpose, the infection being deep down in the tissues. Do not use peroxide for the same reason, and also because some of it will get caught behind the swelling, and with the formation of gas, will give rise to pressure and pain. The object of the treatment is to draw the infection to the surface where it can be dealt with, for it shows no tendency to come there itself. The whole difficulty in the treatment is to get at the infection and thoroughly eradicate it, the tendency for it to lie dormant for long periods (7 days in one case) being an added obstacle.

OPERATIVE INTERFERENCE is found not only to be useless but dangerous as well, for the following reasons :—

(1) It is difficult to locate the position of the abscess in the deeper tissues, because the swelling involves the whole wall of the canal. When the abscess discharges pus it does so through a winding narrow sinus.

(2) If the knife does not strike the abscess, it opens up fresh tissues to infection.

Repeat the treatment given above on the second day. After the second day, the *pure carbolic treatment* should be employed at once, or if the meatus is completely obliterated on the first day, start with this method right away.

Pure Carbolic Treatment. A fine probe covered at the end with a very thin layer of cotton wool is dipped into pure carbolic acid and is used to touch the sides of all vesicles after they have been removed and the pus dried off. If a sinus is found, the exit should be cauterised and the probe passed down it as far as possible without causing bleeding. If, again, no vesicle can be located and at the same time the swelling of the walls of the meatus indicate the presence of a deep-seated abscess, the pure carbolic should be painted over

the most prominent part of the swelling on each wall. Care should be taken to avoid letting the acid overrun its allotted boundaries; this might produce severe burning or might injure the drum. But apart from this, large areas of meatal wall can be safely painted without fear of permanent damage. As much of the ear as possible should be dried carefully, for if the ear is moist the acid will diffuse over the ear and form a strong solution, which will injure the drum. After drying thoroughly, clean part with S.V.R. and allow it to evaporate. Then apply the pure carbolic with the tip of the probe moistened only, and paint the part two or three times till the surface turns white. Then pack the ear with dry wool. This is repeated the second day. On the third day it will be seen that the acid has killed the superficial epithelium, which can be readily removed, and the carbolic can then be applied to the exposed surface.

Gentle pressure should be applied to the swelling, but if pus does not readily appear, the pressure should not be continued, as it will force the pus along the lower planes and thus infect new tissue. If pus comes to the surface, it should be carefully wiped off, and care taken that it does not touch other parts of the meatal wall. Under this treatment the symptoms will rapidly disappear. The swelling goes down quickly, allowing of free access to the deeper portion of the meatus, which should be carefully cleaned each time; the pus will either come to the surface and discharge, or it will be absorbed. After the four applications, it is not as a rule necessary to continue with the carbolic. Treatment should, nevertheless, be continued for another week. The meatus should be cleansed with perchloride and spirit and the ear then packed with wool coated with *Ung. hydrarg. ammon.* and *Ung. acidi boric*, equal parts. Careful inspection should be made for small vesicles or tender spots, so that these can be promptly treated with carbolic.

The following are illustrative cases:—

Case 1. R. 12th October, 1920. Great pain in ear, meatus swollen and inflamed. Tenderness ant. and below swelling of glands in same positions. Drum slightly reddened, but otherwise normal. Temperature 99°.

13th October. Had no sleep previous night on account of pain, which is now very severe. Meatus more swollen, obscuring drum. Temperature 101°.

14th October. Improved. Small blisters along wall of meatus, yellowish-green in colour. (Culture made).

16th October. Improved. One blister seen. (Culture—*B. pyocyaneus*). Patient thought he was cured and did not return till the 22nd October.

22nd October. Intense pain in ear commenced previous day. Three blisters present close to the drum. (Second Culture made).

23rd October. Pain gone. One blister present. (Culture—*B. pyocyaneus*).

25th October. No symptoms. Raw spot at side of blebs.

29th October. No signs of infection. No further recurrence.

Treatment as indicated above.

Case 2. N. 25th June, 1921. Right ear, great pain, one day's duration. Meatus swollen and inflamed. (Culture made).

29th June. Meatus still swollen and painful.

30th June. Pain less, swelling less.

1st July. Still swollen, pain less. (Second sub-culture shows the typical green colour of *B. pyocyaneus*).

2nd July. Great improvement.

4th July. Symptoms nil.

11th July. Pain both ears very acute, one day's duration, meatus in both swollen and inflamed. Yellow blebs present, yellowish-green in colour and very numerous. For the next two days the pain and swelling got steadily worse and patient got little or no sleep. Slight improvement on third and fourth days, but swelling completely blocked meatus.

Treatment. The usual routine as described above was continued for five days when patient went to Java and returned five weeks later with the following history :

Treated for one week and symptoms all disappeared, condition apparently cured. One week later relapse occurred and all the symptoms returned. Treated for two weeks, pain disappeared in both ears and right ear cleared up completely, but discharge and swelling still continued in left ear.

23rd August. Right ear normal, except for slight redness of walls of meatus and opacity of drum. Left ear, walls of meatus so swollen as to be in apposition ; pus present at entrance, seen to be oozing from a small sinus, the mouth of which is blocked by a granuloma ; acute tenderness of wall of meatus, but no pain.

Treatment. Packing with perchloride proving useless, pure carbolic treatment was started on August 26th. Under this the condition gradually cleared up and by September 5th, all symptoms had disappeared.

15th September. A small bleb appeared, showing how tenaciously the infection clings to the walls of the meatus. This was removed and the site touched with pure carbolic. After this no further trouble was experienced and the condition rapidly cleared up and did not return.

This case lasted in all eleven weeks and illustrates the difficulties met with, as well as clearly showing the efficiency of the carbolic treatment. It also shows that it is not safe to conclude that a case is cured, until all symptoms and signs have been absent for at least two weeks.

CONCLUSION

In the tropics, infections of the external auditory meatus are extremely common. I believe I have furnished sufficient evidence to show that 'True Tropical Ear' is a clinical entity, and that *B. pyocyaneus* is the cause of the infection.

TABLE I.

DIFFERENTIAL DIAGNOSIS.

Symptoms	Otomycosis	Other Bacterial	Tropical Ear	Middle Ear disease	Furuncle
Onset.....	Gradual	Gradual	Very acute	Acute	Acute
Pain	Nil	Nil	Very severe	Severe	Very severe
Tenderness ...	Nil	Nil	Whole canal involved very severe Pinna. ant. and suf. glands	Nil	Some tenderness glands; severe the site of ab
Swelling	Nil	Nil	Whole canal involved; very marked. Pinna. ant. and inf. glands	Nil	In canal at site abscess and ma ant. and inf. gl also :
Course	Chronic with repeated recurrence	Short	Short with repeated recurrence		Short, liable to re rence
Appearance ...	Blocked with white cheesy wax	Mild inflammation of wall and drum	Yellow vesicle or meatus may be obliterated by apposition of walls	No swelling of meatal walls	Swelling of absce clearly seen
Deafness	Depends entirely on mechanical blocking of ear	Slight	Depends on blocking of walls by swelling	Marked	Slight
Fever and Malaise	Nil	Nil	Malaise marked fever ranges between 99—101°	Malaise very marked fever to 104°	Malaise marked fe mild
Drum	Very rarely	Unaffected	Slight inflammation only	Bulging of drum and later perforation	Unaffected
Etiology	Fungi	Staphylococcus micrococcus Catarrh, *Bal. coli*, etc.	*Bacillus pyocyaneus*	Staphylococcus usually	Staphylococcus usu
Discharge	Nil	Slight serous	Greenish yellow. Small quantities in later stages, none at first	None at first, and then copious yellow pus	Thick yellow pus wh abscess breaks

EXPLANATION OF PLATE IV.

Fig. 1. Early stage of *Aspergillus glaucus* infection of the ear. Fungus seen growing on the drum and on the walls of the meatus.

Fig. 2. Early stage of *Aspergillus niger* infection of the ear. Fungus seen growing on the walls of the meatus and on the drum.

Fig. 3. Late stage of Aspergillosis. Canal completely blocked with greyish, crinkled material, which is composed of a mass of felt-like fungus and infected wax.

Fig. 4. *Saccharomyces* infection of ear. Walls of meatus unaffected. Drum shows large perforation and two yellow patches.

Fig. 5. *Saccharomyces* infection of ear (later stage). Showing large horse-shoe like perforation of drum.

Fig. 6. *B. diphtheriae* infection of ear. White membrane can be seen growing from the wall of the meatus on to the drum itself.

Fig. 7. 'True Tropical Ear.' 1st stage, showing small, greenish-yellow vesicles on the wall of the meatus. Drum unaffected.

Fig. 8. 'True Tropical Ear.' 2nd stage. Walls of canal red, inflamed and very swollen. Canal almost obliterated by apposition of walls.

Fig. 9. 'True Tropical Ear.' 3rd stage, showing deep-seated abscess, which is discharging pus through a minute sinus. At the mouth of the sinus is seen a small, pouting granuloma.

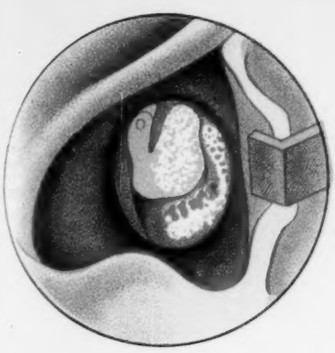
Fig. 10. 'True Tropical Ear.' Canal packed with wool through which the bright green colour of *B. pyocyaneus* shows clearly.

Figs. 11, 12 and 13. Diagrams of Drum in different stages of *Saccharomyces* infection.

Fig. 11. Small perforation and diseased patches devitalized by the Yeast.

Fig. 12. Extended perforation.

Fig. 13. Permanent perforation left after the disease.



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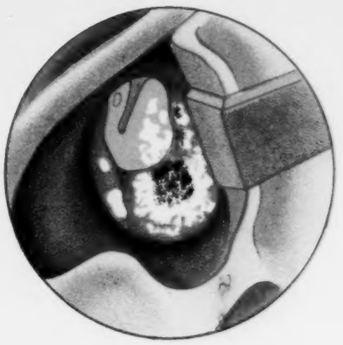

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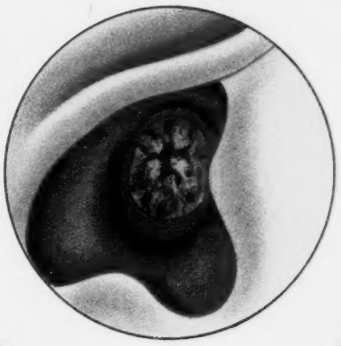

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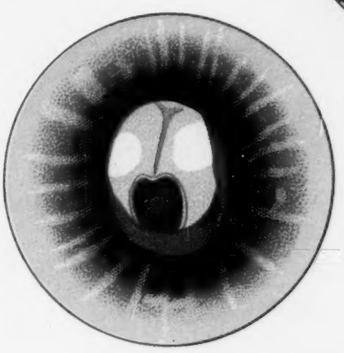

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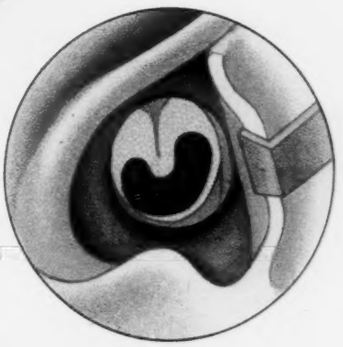

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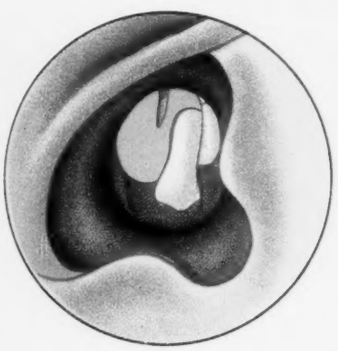

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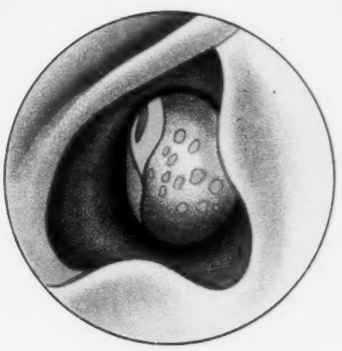

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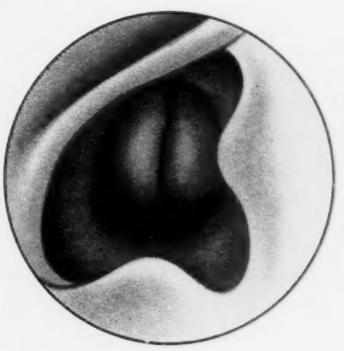

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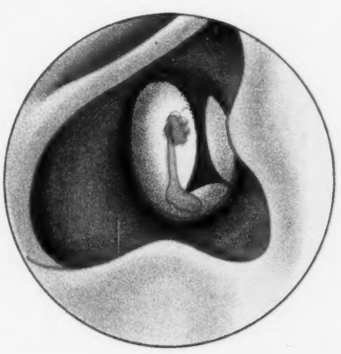

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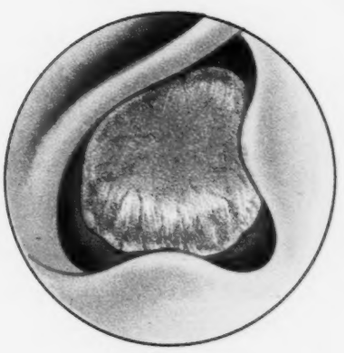

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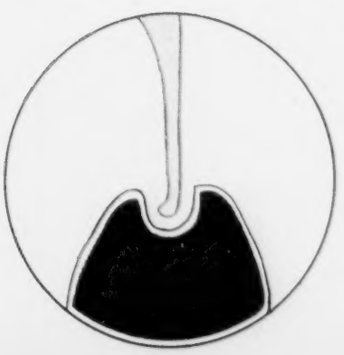

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ON THE FUNCTION OF THE OESOPHAGEAL DIVERTICULA IN THE ADULT FEMALE MOSQUITO

BY

W. REES WRIGHT, B.Sc.

(*Department of Zoology, University College of North Wales, Bangor*)

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The Oesophageal Diverticula are three sacs arising from the posterior portion of the oesophagus of the mosquito; they are present in both sexes. One sac, which is many times larger than the two others, lies ventrally in the thorax and the anterior portion of the abdomen, and opens on the ventral surface of the oesophagus at about the level of the first pair of legs; the distance to which it extends backwards in the abdomen varies according to the amount of food present in either the sac or the mid-gut; thus when the sac is full and the mid-gut empty, the former extends to the sixth or seventh segment, while when the mid-gut is full and the sac empty of food, the latter may be seen as a translucent area in the ventral portions of the first two abdominal segments. The two smaller sacs are dorso-lateral in position, lie in the thorax only, and open into the oesophagus at the same level as the large ventral sac. All three sacs contain a gas in the form of numerous bubbles; Hindle (1914) states that this gas is carbon dioxide produced by certain commensal fungi belonging to the *Entomophtoraceae*.

These organs have been described by a number of authors, the most complete and accurate account being that of Nuttall and Shipley (1903). These authors have also investigated the function of the sacs, and regard them as food-reservoirs. Their paper contains valuable discussions of the descriptions of several previous writers, and of various theories as to the function.

Christophers (1901) describes the organs briefly, and states that

'after feeding . . . . blood is very evident in the mid-gut and even in the calyx-like proventriculus, yet in the oesophagus there is no trace . . . . In a fed mosquito a transparent area is generally to be seen in front of the opaque mass of blood in the abdomen. This transparent area is the abdominal portion of the air-containing oesophageal diverticulum.'

Nuttall and Shipley (1903) describe experiments which they carried out to determine the function of the diverticula. These experiments, in which *Culex pipiens* was chiefly used, consisted in feeding the mosquitoes 'with blood-serum and sugar, either alone or together with carmine or neutral red. Sometimes the feedings took place alternately on coloured and uncoloured solution.' The results of these experiments are summarised in the following Table (Table I). Two experiments* (9 and 10) in which the number of insects used is not stated, are not included in the Table, but are given in Notes A and B below.

TABLE I.

Summary of Nuttall and Shipley's Experiments.

Mosquitoes fed on serum-sugar, either plain or coloured.

	Total	Food sacs	In gut	Remarks
Killed at once	3	3	1	A little in mid-gut of one
Killed after 1 hour	1	1	1	Most in ventral sac
Killed after 24 hours	5	5	2	Moderate amount in mid-gut. Note A.
Killed after 48 hours	5	5	1	Note B.
Fed again after 24 hours	6	6	6	Note C.
Fed again after 48 hours	2	2	2	Note D.

NOTE A. 'Experiment 9. Several insects fed on sugar-carmine-serum, killed after 24 hours showed carmine in intestine down to rectum, besides in ventral sac.'

NOTE B. 'Experiment 10. Several insects, treated as in 9, were killed after 48 hours, there being more carmine in intestine and rectum.'

* The numbers of the experiments refer to their order in Nuttall and Shipley's paper.

Note C. 'Experiment 14. Six insects, fed as in the preceding case (sugar-serum tinged with neutral-red) were fed again after 24 hours on clear sugar serum. The result was very striking. The contents of the ventral sac were coloured red, those of the stomach yellow, so that there could be no doubt but that the second meal had been almost entirely taken up by the stomach.'

Note D. In Experiments 4 and 5 the insects were fed as in 14, the interval being 48 hours. In one ' no bubbles in the much distended ventral sac, which contained carmine. Much aggregated carmine in the stomach.' In the other 'large bubbles and carmine in ventral sac. Little carmine in intestine, which contained some clear serum.'

Most writers subsequent to Nuttall and Shipley follow these authors in regarding the diverticula as food reservoirs. Thus, Patton and Cragg (1913) state that they ' are in fact, true food-reservoirs, as was first shown by Nuttall and Shipley. In mosquitoes killed during the act of feeding they are always found full of blood, while a little later, depending on the rate of digestion and, therefore, on the temperature, the blood is almost entirely confined to the mid-gut.' They point out, however, that the conditions of Nuttall and Shipley's experiments were highly artificial. Hindle (1914), also, regards the sacs as food reservoirs, but notes that the question is ' far from settled.'

In the course of some investigations on the Biology of the British Mosquitoes, the present writer found that although insects which had recently fed on blood were fairly common, he never encountered any which had the least trace of blood in the oesophageal diverticula. The species under investigation were *Anopheles maculipennis*, *Theobaldia annulata*, and *Culex pipiens*, and, at a later date, *A. bifurcatus* and *Aedes detritus*. He commenced experiments with *Anopheles maculipennis*, with the results shown in Table II ; these experiments, which had to be abandoned shortly after their commencement, consisted in feeding the insects on the human subject. At a later date he was able to resume the investigation ; this second series consisted of a repetition of his former work and also of Nuttall and Shipley's experiments using as food an aqueous solution of cane sugar tinged with neutral-red. This second series is summarised in Table III. In connection with the experiments with sugar solution as food, it was observed that the mosquitoes took a far longer time to gorge themselves than they usually take with a blood meal. In this second series of experiments the insects were killed by means

of ether immediately after feeding, and dissected at once. When the gut or sacs were not full of food they were carefully examined under a magnification of at least × 100.

TABLE II

Writer's First Series.

Mosquitoes fed on human subject.

	Total examined	Condition of Diverticula		
		Much blood	Trace of blood	No blood
Gorged, killed at once	14	1	3	10
Disturbed while feeding, killed immediately ...	11	0	3	8
Gorged, killed within 15 minutes	3	0	1	2
Gorged, killed within 60 minutes	2	0	1	1
Total	30	1	8	21

TABLE III.

Writer's Second Series.

Mosquitoes (*Anopheles maculipennis* and a few *Theobaldia annulata*) fed on human subject or on cane sugar—neutral red. All killed immediately after feeding.

	Total	Sacs		Gut	Remarks
		Much food	Trace of food		
Human subject ...	18	...	5	18	
Sugar—neutral red ...	23	23	...	...	
Fresh fruit juice ...	1	1	...	...	A *Theobaldia*, fed on a piece of orange

When we come to consider the various records, we find considerable discordance. Thus the statements of Christophers and of Patton and Cragg are entirely opposed to each other. The writer's own field observations and part of his experimental work favour Christophers' statements, while Nuttall and Shipley's results, and

those of the writer's experiments with 'artificial food,' strongly support the statements of Patton and Cragg.

It is generally agreed that the original food of the Culicidae was plant juices as it still is to-day in the case of the males of all species and the females of many, and that blood is an 'acquired taste.' If we consider the experimental evidence, we must agree that the experiments in which the food-material used most closely approximated to the primitive food of the insects were those of Nuttall and Shipley, and those of the present writer with sugar solution. In these the Oesophageal Diverticula were undoubtedly functional as food-reservoirs. In the case of blood-meals we are dealing with a more recently acquired character, and consequently must not be surprised to find, as we undoubtedly do, that the organs function irregularly.

In an experiment included in Table III, the writer found that fresh fruit juice was taken up into the ventral sac. In this connection it is interesting to note that Blacklock and Carter (1920) found that females of *Anopheles plumbeus* fed on human blood, 'when it was obvious that they had recently partaken freely of the raisin diet provided.' Presumably, the fruit juice was taken up by the diverticula and the blood by the gut.

The writer would suggest as a provisional hypothesis, that the original function of the Oesophageal Diverticula is that of food-reservoirs, but that this function is largely suspended in the case of blood-sucking females.

It has been suggested by some authors that these sacs, being 'air'-filled, would tend to buoy-up the insect when in flight. It is obvious that they would not function so, unless the density of the gases within was less than that of the external atmosphere, and, if Hindle is correct in his statement that the gas present is carbon dioxide, they would tend rather to weigh the insect down.

ACKNOWLEDGMENTS

The writer has much pleasure in taking this opportunity of thanking Professor R. Newstead, F.R.S., and Professor Warrington Yorke, M.D. for valuable suggestions as to the presentation of his results.

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NOTES ON SOME MOSQUITO LARVAE FROM NORTH WALES

BY

W. REES WRIGHT, B.Sc.

(*Department of Zoology, University College, Bangor*)

(*Received for publication* 18 *February*, 1924)

The material which forms the subject of this paper was obtained during the course of some investigations on the breeding-habits of the North Wales Culicidae, the results of which were published in these ANNALS (Rees Wright, 1923).

I. AN UNDESCRIBED LARVAL INSTAR OF *Theobaldia* (*Culicella*) *morsitans* (Theobald). (Fig. 1.)

So far as the writer is aware, the first instar larva of this species has not been described.

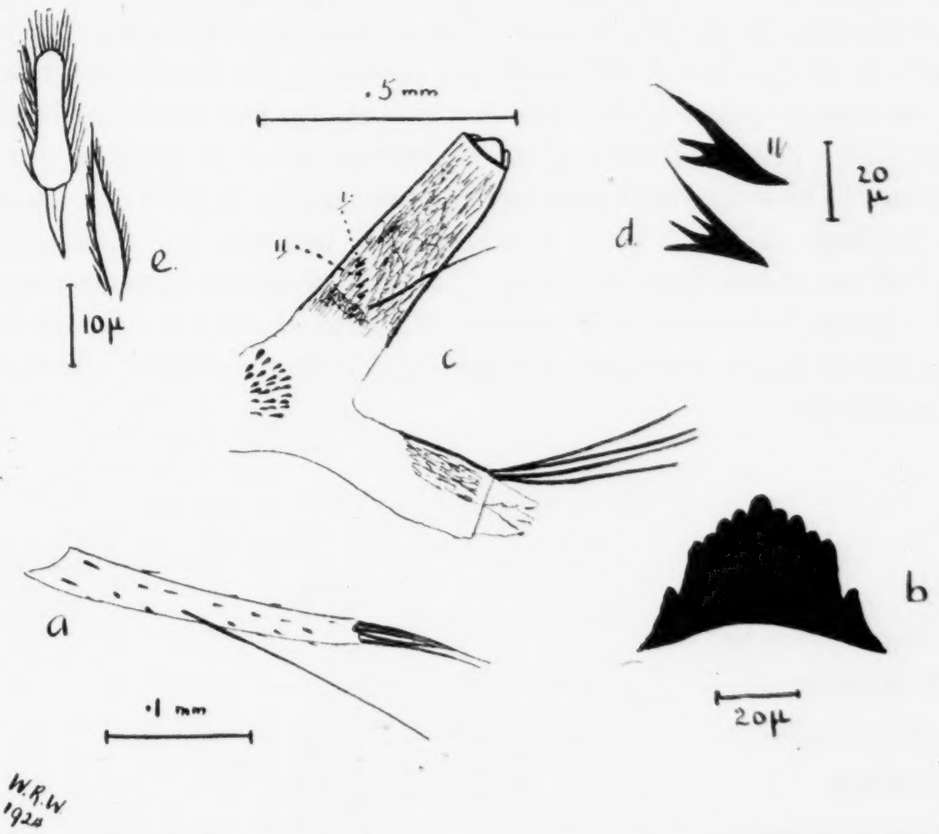

FIG. 1. *Theobaldia morsitans*; 1st Larval Instar. *a*—Antenna; *b*—Mental plate; *c*—Siphon and Anal segment; *d*—Pecten teeth; *e*—Comb-scales.

Head about as broad as long, all hairs simple, with an egg-breaking tooth on the dorsal surface. *Antenna* (fig. 1, *a*) shorter than the head, basal tuft a single hair. *Mental plate* (*b*) triangular, with a large median tooth and four smaller teeth on either side, the outermost slightly separated from the rest. No *anal fin*, four long and stout hairs arise from posterior margin of saddle (*c*). Comb-scales (*e*) few, of two kinds similar to those of the adult. *Pecten* oblique, of four teeth in which the main denticle is decidedly larger than the rest, which are not, however, reduced to small projections on it (*d*). *Tufted hair* single. *Siphonal index* about 3.

Glan-rhyd Reservoir, Pentir, near Carnarvon. 21.9.1922.

II. Differentiation of Larval Instar of *Aedes* (*Ochlerotatus*) *detritus* (Haliday).

Lang (1920) gives a key to the instar of this species; he notes that the characters are not diagnostic 'but should be applied with the reservation that in any one specimen any character may vary beyond the prescribed limits.' The writer obtained some larval pelts of this species which could not belong to the fourth instar, as there were no pupae in the tank in which this species was being bred, but which possessed most of the characters given by Lang for that instar. The writer later obtained the two last larval pelts of a single individual. Table I gives a comparison between the characters found and those given by Lang; Table II gives the extreme range of variation observed in a number of larvae examined. It will be noticed that the characters for the third instar 'overlap' those of the fourth.

Table I.

	Lang (1920)		W.R.W.	
	III	IV	III	IV
Inner post-antennal hair	1	2	2	4
Middle post-antennal hair	1	...	2	2
Outer post-antennal hair	3-4	4+	6	10
Pecten teeth	10-14	18-30	18	23
Tufted hair of Siphon	2-3	4-9	7	10

TABLE II.

	Instar	
	III	IV
Inner post-antennal hair	1-2	1-3
Middle post-antennal hair	2-5	2
Outer post-antennal hair	2-6	2
Pecten teeth ... ... At least	18	...
Tufted hair of siphon	6-7	6-10

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AN *ISOSPORA* OF CIVET CATS

BY

S. ADLER

(*From the Sir Alfred Lewis Jones Research Laboratory, Freetown*)

(*Received for publication 22 February*, 1924)

PLATE V

An *Isospora* was studied in three heavily infected civet cats, *Viverra civetta*—two young animals in which the infection proved fatal, and one adult which was killed for pathological examination.

The animals passed blood and mucus and a large number of oöcysts in their faeces.

The parasite was studied in scrapings of infected mucosa, in sections and in smears. Smears were prepared in the following way : a portion of infected gut was gently washed in water to remove débris, the mucosa was then removed with a scalpel and gently rubbed on the surface of a slide ; prepared in this way, the relationship between the parasite and the epithelial cells is in many instances hardly disturbed. Some of the smears were fixed in Schaudinn's fluid and stained with iron haematoxylin ; others were fixed in methyl alcohol and stained with Giemsa's stain. If the débris is carefully washed away from the infected mucosa, Giemsa's stain gives good results.

DESCRIPTION OF THE PARASITE IN THE HOST

Development is confined to the epithelium of the lower half of the small intestine. Although trophozoites penetrate into the tissue they do not undergo further development there ; their nucleus breaks up into granules and they eventually disintegrate, or they are occasionally engulfed in the lymphocytes (fig. 9).

The merozoites vary in size from 4·0μ to 9·6μ in length by 0·8μ to 2·4μ in thickness. This large variation depends on the number

of merozoites in the schizonts, on the size of the schizonts, and partly on individual variation. Thus in abnormal schizonts containing four merozoites (fig. 19) these may reach 9·6μ in length by 2·4μ in breadth, and on the other hand, in schizonts containing more than eight merozoites (fig. 22) they may be as small as 4μ in length by 0·8μ in breadth. In normal schizonts containing eight merozoites which are by far the commonest (figs. 20 and 21) variations in size occur (4·8μ to 6·2μ in length by 1μ to 1·8μ in breadth). There is no evidence that the large merozoites found in schizonts are differentiated for the sexual cycle. Probably the size of the merozoites in this particular parasite is determined only by the space available for growth inside the schizont and has no sexual significance. The merozoites inside the schizont are usually slightly bent and are pointed at one extremity.

The nucleus of the merozoite is relatively large ; it has a distinct nuclear membrane and contains a large karyosome which may lie in the centre of the nucleus or it may be attached to a portion of the nuclear membrane.

Trophozoites are found inside vacuoles in the infected cell, the pointed end of the parasite being attached to a point in the wall of the vacuole (figs. 1 and 2). Double infection of cells was frequently seen and occasionally three merozoites were found parasitising one cell.

In cases of multiple infection, two trophozoites may be found in one vacuole (fig. 16), or each parasite may lie in its own vacuole (fig. 17).

The trophozoites grow inside their vacuoles and are usually gregariniform in shape. The variations found in the shapes of the trophozoites are shown in figs. 3 to 8.

When the trophozoite has attained a size of 9·6μ to 11·1μ in length by 3·2μ to 3·7μ in its thickest part, nuclear division takes place. This is accomplished in the following manner :—The karyosome divides by simple fission, and the two daughter karyosomes move to opposite ends of the nucleus ; between them a spindle stretches, on which two plates of chromosomes form ; these move towards the daughter karyosomes and the nuclear membrane then divides by constriction between the two karyosomes, two daughter nuclei being formed. The schizont maintains its gregarine form

till it contains eight nuclei ; it then becomes round, the protoplasm segments and eight merozoites are formed. The schizont may or may not contain a residual body. Abnormal schizonts are found which contain more or less than eight merozoites (figs. 18, 19, 22). Schizonts vary from 10μ in diameter (containing four merozoites) to 18·4μ. Normal schizonts containing eight merozoites are generally about 16μ in diameter.

MICROGAMETOCYTES

As previously stated it was not found possible to distinguish the merozoites destined to become microgametocytes, but after several nuclear divisions have occurred, it is easy to distinguish the microgametocyte from the schizont, for it tends to become round or elliptical when it is only 6μ in diameter and it then contains about thirty small round nuclei (fig. 30).

When the growing microgametocyte becomes sufficiently large it is possible to see that nuclear division takes place by mitosis (fig. 32). A stage is reached when nuclear division ceases and the microgametocyte then contains a large number of irregular compact nuclei (fig. 33) ; these become round and from each nucleus a small rod of chromatin protrudes (fig. 34). The nucleus then becomes falciform and finally microgametocytes develop (fig. 35).

The completely developed microgametocyte varies from 16μ to 24·7μ in diameter and contains one, or more commonly two residual bodies round which the microgametes are grouped. There are usually about two hundred microgametes in one fully developed microgametocyte. The microgamete has two flagella and measures about 10μ from the tip of one flagella to the other.

THE MACROGAMETE

The young macrogamete is easily recognisable, for it becomes spherical early in its development, when only 7μ in diameter. At this stage it is very striking, because of its large round nucleus which takes up half the diameter of the parasite (figs. 23 and 24). The nucleus of the young macrogamete is the same in size as well as structure as that of the mature macrogamete. Deeply staining

granules appear in the protoplasm round the nuclear membrane and as the parasite grows, these granules diminish in number and are disseminated throughout the protoplasm. The nucleus of the host cell becomes attached to and often drawn over the vacuole containing the macrogamete (figs. 26 and 28), but this is not absolutely typical of the macrogamete, for it is also occasionally seen in microgametocytes and schizonts.

The protoplasm of the macrogamete consists of a very fine mesh containing small refractile granules. The mature macrogamete attains almost the same size as the oöcyst. Macrogametes were found to be about thirty times as numerous as microgametocytes.

Fertilisation takes place inside the host cell. As many as nine or ten microgametes were observed inside one macrogamete (fig. 29).

After fertilisation the hard wall of the oöcyst is formed. The protoplasm of the oöcyst contains coarser granules than the macrogamete. In addition to the numerous small refractile granules, the protoplasm contains from one to five larger refractile bodies and these are extruded during the first division of the oöcyst (figs. 36 and 37) forming an oöcystic residue which disappears before development of the oöcyst is completed. The first division takes place inside the invaded epithelial cell or in the gut. Oöcysts containing two sporoblasts are found in the gut with the remains of the nucleus and protoplasm of the invaded cell attached to them. No further development takes place inside the host and the oöcysts containing two sporoblasts are passed in the faeces.

A number of oöcysts are passed with the protoplasm unsegmented but they are probably non-fertilised macrogametes which have become encysted, for they do not appear to develop further outside the body of the host.

DEVELOPMENT OUTSIDE THE HOST

After twenty-four hours the sporoblasts have a sporocyst round them and become spores, each spore containing two masses of protoplasm and a residual body. The spore measures 12·5μ to 15·2μ in length by 8μ to 11μ in breadth; the residual body at this stage measures 6μ by 10μ.

After three days' development is completed, each spore then contains four sporozoites and a residual body ; the sporozoites are sickle-shaped and measure 9·5μ to 11·2μ in length by 2·8μ in their thickest part ; the residual body is more diffused than in the previous stage, so that it is impossible to give definite measurements. After standing for two or three weeks, the residual body in the spore is reduced to a few granules.

The oöcysts vary in size from 19μ to 27·5μ in length by 15·2μ to 24·7μ in breadth, the commonest size being 22·8μ by 19μ, and the extremes 15·2μ by 19μ and 24·7μ by 27·5μ being only two per cent. of the total number measured. They thus approximate in size to, but are slightly larger than *Isospora rivoltae*, Grassi, 1879 (15μ to 20μ by 20μ to 24μ) in English dogs (Wenyon, 1923).

IDENTITY OF THE PARASITE

The oöcysts are approximately the same size as *Isospora rivoltae*, Grassi, 1879 ; but the *Isospora* of civet cats has certain features which distinguish it from the former.

(1) Its oöcysts are passed containing two sporoblasts whereas in *I. rivoltae* the oöcysts are passed unsegmented.

(2) It was not found possible to infect cats and kittens, and young dogs by feeding with heavily infected material. Two cats, three kittens and two young dogs were used for the experiment, and they failed to become infected within an observation period of twenty-eight days.

The *Isospora* of civet cats is therefore probably not the same species as *Isospora rivoltae* and I propose the name *Isospora viverrae*, n.sp., for the parasite.

PATHOLOGY OF THE INFECTION

On section, the infected parts of the small intestine showed that in the basal parts of the villi few cells were infected, whereas the distal parts were heavily infected. A similar observation was made by Wenyon in the case of *Isospora felis*, Wenyon, 1923. In many villi the distal part was entirely denuded of the epithelium. The sub-epithelial tissue in infected areas was markedly hyperaemic.

In spite of the heavy infection and the changes of the sub-epithelial tissues no ulceration was observed. In this connection it is interesting to note that during 1922 and 1923 about thirty specimens of *Viverra civetta* were examined in the Sir A. L. Jones Laboratory, in Freetown, and in no case was ulceration of the small intestine noted. It seems probable, therefore, that regeneration takes place from the epithelium of the basal and comparatively unaffected portions of the villi.

An interesting fact was observed, namely, that in heavily infected parts of the small intestine islands were found which on serial section appear to be free from infection. It is hardly likely that the cells of these portions surrounded by heavily infected areas were not subjected to invasion by merozoites. A more feasible explanation is, that these islands of healthy mucosa had been infected and the villi denuded of epithelium distally; regeneration from the healthy epithelium situated at the base of the villi then took place and the regenerated areas became immune to further infection, i.e., we are dealing with a case of local and acquired immunity to a protozoon.

In the two young animals, numerous schizonts as well as sexual forms were found, whereas in the adult animal schizonts were comparatively few and were only found after prolonged search; it is probable, therefore, that the adult animal was on the eve of a spontaneous cure in spite of a heavy infection.

SUMMARY AND CONCLUSIONS

An *Isospora* was studied in three heavily infected civet cats.

The asexual and sexual cycles within the host are described.

Oöcysts containing two sporoblasts were found in the intestine and in fresh faeces.

The oöcysts measured 19μ to 27·5μ in length by 15·2μ to 24·7μ in breadth.

The development of the oöcysts outside the body of the host is described.

Cats and dogs fed on heavily infected material failed to become infected within an observation period of twenty-eight days.

The name *Isospora viverrae*, n.sp., is proposed for the *Isospora* of civet cats.

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EXPLANATION OF PLATE V

Figs. 1, 2. Trophozoite inside vacuole.
Figs. 3-8. Various forms of trophozoites.
Fig. 9. Trophozoite inside lymphocyte.
Figs. 10-12. Trophozoites showing nuclear division.
Figs. 13-15. Schizogony.
Fig. 16. A vacuole containing two parasites, a merozoite and one young schizont.
Fig. 17. One cell with two vacuoles each containing a parasite.
Fig. 18. Schizont with four small merozoites.
Fig. 19. Schizont with four large merozoites and a residual body. Note large size of merozoites and residual body.
Figs. 20, 21. Schizonts with eight merozoites.
Fig. 22. Schizont with 13 merozoites showing.
Figs. 23-28. Showing development of macrogamete. Note in 26, 27 and 28, application of nucleus of host cell to wall of vacuole. Note in young macrogametes the large size of the nucleus.
Fig. 29. Macrogamete invaded by a number of microgametes.
Fig. 30. Young microgametocyte.
Fig. 31. Later stage of microgametocyte. Note relationship of the nucleus of host cell to vacuole.
Fig. 32. Later stage of microgametocyte showing mitotic figures and irregular masses of chromatin.
Fig. 33. Still later stage of microgametocyte showing solid masses of chromatin.
Fig. 34. Still later stage of microgametocyte. From each mass of chromatin a small process protrudes.
Fig. 35. Still later stage showing falciform nuclei and one residual body.
Fig. 36. Oöcyst as found in mucosa.
Fig. 37. Oöcyst as found in mucosa and passed in fresh faeces.
Fig. 38. Fully developed oöcyst. (Drawn from material three months' old, hence the residual bodies are small) × 1250.

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ORNITHODORUS MOUBATA, MURRAY, IN RELATION TO RELAPSING FEVER IN THE GOLD COAST

BY

A. INGRAM, W.A.M.S.

(*Received for publication 27 February*, 1924)

The carrier of African tick fever *Ornithodorus moubata*, which Brumpt (1922) definitely states does not convey *Sp. recurrentis*, appears to be absent from the Gold Coast and consequently cannot have played any part in the recent outbreak of relapsing fever at Accra. An opportunity, however, was given of testing the efficiency of this species as a carrier of the strain of spironemata causing the epidemic in the Gold Coast, when a consignment of *O. moubata*, hailing originally from Rhodesia, was received in September at Accra from the Liverpool School of Tropical Medicine, thanks to the courtesy of Dr. J. W. Scott Macfie.

The ticks were kept unfed in the incubator for ten days after their arrival, so that they might recover from an apparent state of lethargy induced probably by the conditions encountered on their journey. On the 14th September eighteen of the ticks were placed on a pouched rat, *Cricetomys gambianus*, Waterhouse, which had been inoculated with blood from a case of relapsing fever and was showing a heavy infection with spironemata. Almost all the ticks employed were recovered in an engorged condition the next morning and were despatched at once to Liverpool, where they arrived safely, but, according to information received from Dr. Macfie, failed to convey infection to two rabbits and a monkey upon which they fed. The remaining ticks of this consignment, sixteen in number, were divided into two lots, which were placed upon two different pouched rats that had been inoculated on the 7th September from a case of relapsing fever and which showed numerous spironemata in their blood. On the 15th September, the morning after they had been

placed upon the rats these ticks were returned to the incubator, where they were kept until the 2nd October. On this date, all the surviving ticks which had fed on the two infected rats on the 14th September, were placed upon a clean pouched rat. Next morning, however, as only one or two of them seemed to have fed they were again returned to the incubator and the feeding was repeated upon the same rat on the 16th and 30th October by which time the number of ticks had been reduced to four owing to the propensity of the rat to devour them. Thick films of blood from this rat were examined daily from the beginning of the feeding experiments till the 22nd November but spironemata were never found in them and preparations made from the body fluid of the four surviving ticks, which had presumably fed on the rat on one of the occasions on which it was exposed to their attack, failed to show spironemata.

On the 30th November eleven specimens of *O. moubata* from a second consignment, kindly forwarded by Dr. Macfie, were placed on a pouched rat which was showing spironemata abundantly in its blood. Only eight of these ticks, of which five were engorged, were recovered on the following morning and were placed in the incubator. On the 4th December, fourteen fresh ticks were placed on the same rat, which was still showing a few spironemata in its blood; all of these ticks, eight being engorged, were recovered on the morning following and placed in the incubator.

Seven of the ticks, which had been placed on the infected rat on the 30th November, were placed on a clean pouched rat on the 13th December. Unfortunately, only two of them could be found the next morning and neither appeared to have fed. The blood of this rat never showed spironemata in thick films which were examined daily till the end of December.

On the 20th December the ticks which had been placed on the infected rat on the 4th December were placed on another clean pouched rat; eleven of them were recovered on the following morning and were returned to the incubator, several being in an engorged condition. As this last rat experimented with did not show spironemata in thick films of its blood examined daily up to the 4th January, it was on that date again exposed to the bites of fifteen *O. moubata* which had fed previously on infected *C. gambianus*, yet no spironemata were found after this second exposure to the

attacks of ticks, which had every opportunity of becoming infected, although the blood of the rat was examined daily until the 31st January.

It would appear from these feeding experiments that *Ornithodorus moubata*, Murray, is not an efficient carrier of the spironemata causing the recent epidemic of relapsing fever in the Gold Coast.

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THE RELATIONSHIP BETWEEN THE *ASCARIDS* OF MAN, PIG AND CHIMPANZEE

BY

H. THORNTON

(*From the Parasitology Department of the Liverpool School of Tropical Medicine*)

(*Received for publication* 1 *March*, 1924)

The material available for examination was :—

(1) Numerous specimens of *Ascaris lumbricoides* obtained by Dr. J. W. S. Macfie from Asylum patients at Accra.

(2) Numerous specimens of *Ascaris suilla* from pigs killed in the Liverpool Abattoir.

(3) A few *Ascarids* obtained from a chimpanzee (*Anthropopithecus* sp.).

Attempts were made to establish any difference especially in regard to the lips, shape of the pulp and number and position of the cephalic papillae. The tails of the male worms were compared

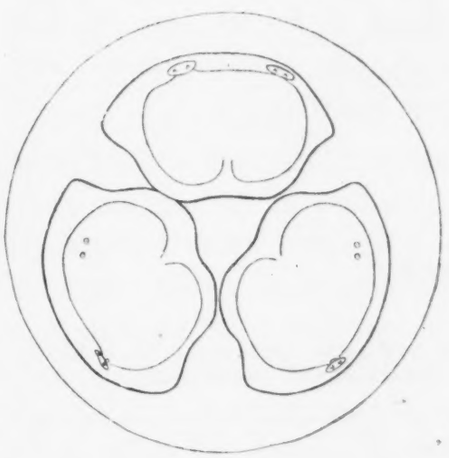

FIG. 1. *Ascaris lumbricoides.* Head, anterior view.

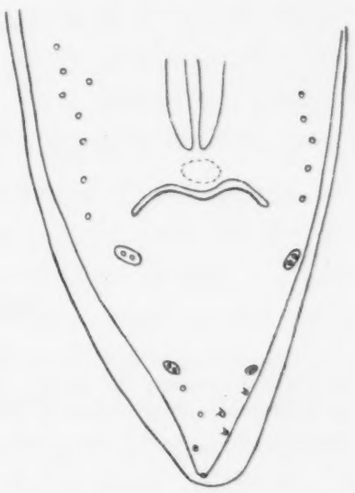

FIG. 2. *Ascaris lumbricoides.* Post-extremity of male, ventral view.

as regards the mode of termination and the number and distribution of the papillae on the ventral surface. The spicules in the male were examined and in the female the position of the genital opening.

On the dorsal lip of the head are two large double papillae; on each of the sub-ventral lips is one large double papillae lying towards the ventral aspect and, more laterally, two small papillae lying close together. This arrangement exists in worms from all three sources. The pulp of the lips appeared to show no difference in the various worms.

The tail of the male terminates in a small mammillate projection. It varies somewhat in shape, but such variations occur not only in worms from different sources, but in worms from the same host. The number and position of the postanal papillae in the male is constant. There are seven pairs, the two anterior being double, and of these the one nearer the cloaca is always the larger. The remaining three pairs are single and arranged on each side in the form of a triangle with the apex directed inwards. Immediately in front of the cloaca is a large cushion-like structure. The preanal papillae are irregular, but in worms from all three sources there are at least 50 pairs. The spicules are broad, flattened dorso-ventrally and non-alate, no differences being found in the worms from the various sources.

Morphologically, then, the *Ascaris* of the pig and of the chimpanzee cannot be distinguished from the *Ascaris* of man, and should, therefore, be designated *Ascaris lumbricoides*, Linn (1758).

These observations agree with those of Baylis and Daubney (1922), who examined and compared the *Ascaris* from man, orang-utan, Indian wild pig and various squirrels.

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ASCARIS LUMBRICOIDES CAUSING FATAL LESIONS IN A CHIMPANZEE

BY

A. W. N. PILLERS

(*Received for publication 7 March*, 1924)

On June 6, 1922, I conducted a post mortem examination on a young female chimpanzee, which had died soon after importation into this country. There was no satisfactory clinical history. After being listless for a few days, she had been found dead one morning; the autopsy was made about twenty-four hours after death. The body was moderately well nourished. The face, ears and those areas of the skin not densely covered with hair, were distinctly icteric. A careful examination of all the cavities and their contained organs showed that gross abnormalities were confined to the small intestine and liver.

The duodenal mucous membrane was discoloured by numerous dark particles—presenting an appearance similar to that of a moderately anthracosed lung. The remainder of the intestinal mucous membrane was slightly inflamed. In the lumen were sixty-three large round worms ranging in length from 6·5 cms. in the case of males to 15·75 cms. in females. These on examination proved to be *Ascaris lumbricoides* (*vide* Thornton, 1924). The liver weighed about 6 ozs. The right or main lobe contained three abscesses, each about the size of an ordinary marble and in each of them was a degenerated worm measuring about 9 cms. in length. In the left lobe was a partly decomposed female worm about 13 cms. in length, bent on itself like a hair pin, each limb running a tortuous course in what appeared to be dilated bile ducts. At the junction of the hepatic and cystic ducts was a considerable dilatation which continued down the ductus choledochus for a short distance. In the sac so formed were six partly decomposed worms which varied in length from 5 to 7 cms. Two were bent on themselves, but the

others were not. There were thus four worms in the liver and six in the duct, making with the sixty-three in the small intestine a total of seventy-three.

The result of the autopsy leads one to conclude that the animal died as a result of the infestation with *Ascaris lumbricoides*, and that death was due more particularly to those worms which had gained the liver, apparently by way of the bile duct.

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THE ACTION OF THE SALIVARY SECRETION OF MOSQUITOS AND OF *GLOSSINA TACHINOIDES* ON HUMAN BLOOD

BY

WARRINGTON YORKE

AND

J. W. S. MACFIE

(*Received for publication 7 March*, 1924)

Whilst conducting experiments with malaria sporozoites from the salivary glands of *Anopheles maculipennis*, we were much impressed by the extraordinary degree of agglutination of the erythrocytes which immediately occurred on mixing an emulsion of salivary gland with citrated human blood. On looking through the literature, we were surprised to find that apparently the only record of this phenomenon is that of Cornwall and Patton (1914), who state that the salivary secretion of *A. rossi* and *A. jamesi* produced strong and immediate agglutination of the red corpuscles of human blood and prevented the blood from clotting. Experiments were undertaken to investigate this subject and to contrast the action on human blood of the salivary secretion of female *A. maculipennis* with that of female *Culex pipiens*, *Theobaldia annulata*, *Stegomyia fasciata* and *Glossina tachinoides*.

In all experiments, the emulsion of salivary glands was obtained by breaking up as finely as possible with needles the entire salivary glands from a single insect in 0·035 c.c. of physiological saline solution, and the citrated blood was obtained by adding three volumes of human blood to one volume of a 1 per cent. solution of sodium citrate in physiological saline solution; one volume of the salivary gland emulsion was then mixed with two volumes of the citrated blood solution and the mixture drawn up into a capillary tube of rather wide bore.

In the case of *A. maculipennis* complete and immediate agglutination of the erythrocytes was observed, whereas in the case of *Culex pipiens*, *Theobaldia annulata*, *Stegomyia fasciata* and *Glossina tachinoides*, respectively, no trace of agglutination of the erythrocytes was seen, even after standing an hour at either 37° C. or laboratory temperature. The experiments, which were repeated several times, were performed with blood from two normal individuals and gave constant results.

Observations were next made in order to obtain some idea of the concentration of the agglutinin in the salivary gland emulsion of *A. maculipennis*. For this purpose the original emulsion containing the complete salivary glands of one mosquito in 0·035 cc. of saline solution was diluted 4 times, 8 times, 16 times, 32 times, 64 times and 128 times, respectively, and as in the previous experiments one volume of the various dilutions mixed with two volumes of citrated blood. Complete and immediate agglutination of the red cells was observed up to a dilution of 32-fold; with the 64-fold dilution there was a considerable immediate agglutination which became complete after standing a little time, while in the case of the 128-fold dilution the amount of agglutination observed was but slight. These observations show clearly that haemagglutinin is present in the salivary glands of *A. maculipennis* in very great concentration.

The agglutination of red cells due to the salivary secretion of *A. maculipennis* takes place about equally well at 37° C. as at laboratory temperature of about 15° C.

Heating the emulsion of salivary gland of *A. maculipennis* for half an hour at 56° C. completely destroys the agglutinin. Further experiments showed that exposure to a temperature of 50° C. for half an hour also completely destroyed the agglutinin, whilst heating to 45° C. for a similar period, produced some slight diminution in its intensity. The haemagglutinin in the salivary secretion of *A. maculipennis* is hence very thermolabile and is destroyed at comparatively low temperatures. When a mixture of emulsion of salivary glands of *A. maculipennis* and blood—the red cells of which are thus completely agglutinated—is heated at 60° C. for two minutes, it was found, on discharging the blood on a glass slide, that the agglutinated masses of red corpuscles had completely dissociated

and no longer re-agglutinated when the temperature fell to 15° C. Heating the mixture to 50° C. for fifteen minutes partially destroyed the agglutinated masses, whilst heating at the same temperature for thirty minutes caused complete disappearance of the agglutination.

The haemagglutinin present in the salivary glands of *A. maculipennis* differs, therefore, in certain respects from the autoagglutinin present in the blood of certain normal animals, and more markedly in the blood of these animals when suffering from certain infections, e.g., trypanosomiasis: firstly, in that the salivary gland agglutinin acts about equally well at 37° C. as at low temperatures, and secondly, in that it is very thermolabile, being destroyed at 50° C.

If the salivary gland emulsion of *A. maculipennis* is allowed to dry at either 15° C. or 37° C. and the residue re-dissolved in a volume of fluid equal to the original volume of the emulsion, it is found that on adding two volumes of citrated blood no agglutination takes place: the agglutinin is thus destroyed by dessication.

Adopting the same technique, it was found that the salivary secretion of *A. maculipennis* agglutinated strongly, but not quite so intensely as in the case of man, the red blood corpuscles of the donkey, rabbit and dog, but had no action on those of the mouse, guinea-pig and monkey (*Cercopithecus* sp.). The salivary secretion of *Stegomyia fasciata* had no agglutinating action on the red corpuscles of any of these animals.

Emulsions of the stomach and of the ventral oesophageal diverticulum of *A. maculipennis* failed to produce any agglutination of human erythrocytes.

No evidence of haemolysis was observed in any of the experiments performed with the salivary gland emulsion of *A. maculipennis*, or any of the other mosquitos, or of *Glossina tachinoides*.

Experiments were finally undertaken with *A. maculipennis*, *Stegomyia fasciata* and *Glossina tachinoides* to determine whether the salivary gland emulsion exerted any inhibitory action on the coagulation of human blood. The salivary gland emulsions were made as described above, the complete glands of one insect being emulsified in 0·035 cc. of physiological saline. One volume of the emulsion was then mixed with two volumes of blood as it escaped from the freshly-punctured finger and after mixing thoroughly,

drawn up into a capillary tube of rather wide bore and placed in a water bath at 37° C. The control tubes containing one volume of physiological saline and two volumes of blood were invariably found to have clotted after five minutes, so that when the contents of the tube were blown out on to a glass slide, a long worm-like red clot was extruded : this red cylindrical mass could not be disintegrated by stirring with a needle or by warming to 60° C., as would be the case if the red cells were merely agglutinated. The salivary secretion of *Stegomyia fasciata* had no effect on the coagulation, the blood having clotted as in the controls within five minutes ; the salivary gland emulsion of *A. maculipennis* merely delayed the coagulation of the blood, no clot being observed after five minutes, but a distinct clot was found after fifteen minutes. In the case of *Glossina tachinoides*, however, the anticoagulating power of the salivary gland emulsion was much more pronounced, no coagulation of the blood being observed after standing at 37° C. for thirty minutes and little, if any, even after twelve hours.

In view of the above observations one is led to enquire what happens to the blood which is drawn into the mosquito stomach on feeding. It was found that the blood in the stomach of *A. maculipennis* two hours after feeding on a human being, exhibited complete agglutination of the erythrocytes, but was unclotted, whereas that in the stomach of *Stegomyia fasciata* a similar time after feeding, exhibited no agglutination of the erythrocytes, but was quite definitely clotted. This suggests that when the mosquito feeds, salivary secretion is first poured out into the wound and then partly withdrawn with the blood into the stomach. The fact that sporozoites were found free amongst the blood in the stomach of an infected *A. maculipennis* two hours after feeding, is additional evidence in support of this; as the mosquito had been infected over five weeks before the meal in question, the sporozoites found amongst the blood in the stomach could not have been contaminations from oöcysts in the stomach—and none were found in the stomach on dissection—but must apparently have had their origin in salivary secretion which, after being discharged into the wound, had been withdrawn with the blood into the stomach. Furthermore, a little blood is occasionally to be found in the ventral oesophageal diverticulum of an *A. maculipennis* when examined an hour or two after feeding

and sporozoites have been found amongst the blood in this situation, a couple of hours after feeding.

We can offer no explanation of the function of the haemagglutinin or anticoagulin in the salivary secretion of *A. maculipennis*. So far both these bodies have been shewn to be present in the salivary secretion of all the *Anopheles* examined, viz., *A. rossi*, *A. jamesi* and *A. maculipennis*, but both are absent in that of *Stegomyia fasciata*, whilst in *Glossina tachinoides* the salivary secretion contains a powerful anticoagulin, but no agglutinin.

SUMMARY

1. An emulsion of the salivary glands of *A. maculipennis* powerfully agglutinates the erythrocytes of human blood and, less strongly, those of the donkey, rabbit and dog ; it has no agglutinating action on the red corpuscles of the mouse, guinea-pig or *Cercopithecus* sp. Emulsions of the stomach and of the ventral oesophageal diverticulum exerted no agglutinating action.

2. The haemagglutinin is thermolabile being destroyed by heating to 60° C. for two minutes or to 50° C. for thirty minutes, and the agglutinated red cell masses dissociate completely under these conditions.

3. Similar salivary gland emulsions of *Culex pipiens*, *Theobaldia annulata*, *Stegomyia fasciata* and *Glossina tachinoides* do not agglutinate human red cells, nor does the salivary gland emulsion of *Stegomyia fasciata* agglutinate the red cells of any of the animals mentioned above.

4. No haemolysin was detected in the salivary gland emulsion of any of these insects.

5. The salivary gland emulsion of *Stegomyia fasciata* exerted no anticoagulating action on human blood, whilst in the case of *A. maculipennis* and of *Glossina tachinoides*, the anticoagulating action was quite definite.

6. The blood in the stomach of *A. maculipennis* two hours after feeding on a human being was found to exhibit complete agglutination of the erythrocytes, but to be unclotted, whilst that in the stomach of *S. fasciata*, a similar period after feeding on man, exhibited no agglutination, but was coagulated.

7. This indicates that the salivary secretion is in part withdrawn into the stomach with the blood when the mosquito feeds; further evidence in support of this is the fact that sporozoites were found intermingled with the blood in the stomach and ventral oesophageal diverticulum of infected *A. maculipennis* examined shortly after feeding.

REFERENCE

CORNWALL, J. W., and PATTON, W. S. (1914). Some Observations on the Salivary Secretions of the Commoner Insects and Ticks. *Ind. Journ. Med. Res.* Vol. II, p. 569.

ADDENDUM

Since writing the above, we have had an opportunity of examining the reactions of the salivary secretion of *A. bifurcatus* and find that it neither causes agglutination of human erythrocytes nor does it delay coagulation of human blood. In these respects, therefore, *A. bifurcatus* differs strikingly from *A. maculipennis*, *A. rossi*, and *A. jamesi*.

ON THE CLASSIFICATION OF THE CESTODE GENUS *MONIEZIA* (BLANCHARD 1891)

BY

G. THEILER

(*From the Parasitology Department of the Liverpool School of Tropical Medicine*)

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The investigation, of which the present article is the outcome, was suggested to me by Professor Warrington Yorke, who kindly put all the material of the Liverpool School of Tropical Medicine at my disposal. I wish here to tender my thanks to him, and also to Mr. Southwell for allowing me to make use of his prepared slides, as well as for his friendly assistance and advice.

The museum collection contained material from cattle, sheep, and goats from Port Said, Malta, Accra, Bâle, and different localities in England. As most of this material was fragmentary and as there could thus be no certainty that the contents of any one bottle originally belonged to one worm only, it was decided to procure fresh material. This was obtained from the Liverpool Abattoirs during the months of May to September, 1923, and only entire worms were kept. All these were preserved and cleared in the same way, in order that so far as possible the differences due to preservation, etc., might be avoided.

It has recently become customary, when examining large cestodes, to select small portions from various positions of the strobila and to identify the species on the data so obtained (Sauter). This rough and ready method is very convenient and may be sufficient in old and established genera, where the differences between the various species are well-defined. But in the genus *Moniezia* it is apt to lead to confusion. The examination of entire worms—a most laborious

proceeding—enables one not only to see the variations present in different specimens, but also variations which may be present in any one worm. In this way, I have been able to show that several differences which were looked upon as specific may, and actually do occur in a single individual.

HISTORICAL

Blanchard in 1891 created the genus *Moniezia* with the following diagnosis :—

'Corps lancéolé en avant, anneaux serrés, beaucoup plus larges que longs, avec deux pores sexués, opposés,'

and included in it the following eleven species :—

M. alba, Perroncito 1878
M. benedeni, Moniez 1874
M. denticulata, Rudolphi 1804
M. expansa, Rudolphi 1810
M. festiva, Rudolphi 1819
M. goezei, Baird 1853
M. leuckarti, Riehm 1881
M. marmotae, Fröhlich 1862
M. neumanni, Moniez 1891
M. nullicollis, Moniez 1891
M. pectinata, Göze 1782

Moniez (1891) included *T. ovilla*, Riv. 1878, and *Thysanosoma actinioides*, Diesing 1834, in this new genus.

Stiles and Hassall (1893) emended Blanchard's definition as follows :—

'Head without hooks ; segments generally broader than long and longer than thick ; end segments shewing a tendency to become longer and narrower. Two full sets of genital organs, with two uteri and two lateral pores in each segment. On the right side the vagina is ventral, cirrus dorsal ; on the left side vagina dorsal, cirrus ventral. Dorsal canal lies dorso-median of the ventral canal. Genital canals cross the longitudinal canals and nerves dorsally. Interproglottidal glands generally present. Calcareous bodies absent from parenchyma. Eggs with well-developed pyriform body.'

Type species *M. expansa* (Rudolphi 1810) Blanchard 1891.

Stiles and Hassall included in this genus eight species which fell into three groups :—

(*a*) *Planissima* group, characterized by the linear arrangement of the interproglottidal glands.

M. planissima
M. benedeni
M. neumanni

(*b*) *Expansa* group, characterized by the saccular arrangement of the interproglottidal glands.

M. expansa
M. oblongiceps
M. trigonophora

(*c*) *Denticulata* group, in which the interproglottidal glands are absent.

M. denticulata
M. alba

As *Moniezia* they include five of Blanchard's original species and further describe three new species. Later, Stiles classifies as *Cittotaenia* the double-pored leporine forms, which differ from *Moniezia* in the following particulars :—

'Vagina ventral to the cirrus-pouch on both sides of the segment; interproglottidal glands absent; generally one, but in some cases two, simple transverse tubular uteri in each segment; uterus generally possesses simple, proximal and distal diverticula. Eggs with a well-developed pyriform body, the horns of which are long, generally filamentous and cross each other. Type-species *Cittotaenia latissima*, Riehm 1881 = *Cittotaenia denticulata* (Rudolphi 1804), Stiles and Hassall 1896.'

In this genus Stiles includes four of Blanchard's original *Moniezia*, namely :—

M. denticulata = *Cittotaenia denticulata*
M. goezei = *Cittotaenia denticulata*
M. marmotae = *Cittotaenia marmotae*
M. pectinata = *Cittotaenia pectinata*

M. nullicollis. Stiles does not consider this to be a well-established species, as knowledge of its anatomical details is lacking.

M. festiva. He mentions this species as being recorded as a *Moniezia* by Blanchard. Nybelin (1917) has since re-examined Rudolphi's original specimen and has made it the type species of a new genus *Hepatotaenia*, closely allied to the genus *Cittotaenia*.

Fuhrmann (1902) placed the double-pored avian cestodes—the genus *Paronia* of Diamare 1900—into the genus *Moniezia*. They

were left here until 1918, when Fuhrmann accepted the genus *Paronia*. This genus differs from *Moniezia* in the arrangement of its genital ducts; in the structure and origin of its uteri, and in the absence of a pyriform apparatus in the egg.

Since the revision of the adult cestodes of cattle, sheep and allied animals by Stiles and Hassall, several new *Moniezia* have been described, namely:—

M. rugosa (Diesing 1850), Lühe 1895, in *Ateles hypoxanthus*.

M. amphibia, von Linstow 1901, in *Hippopotamus amphibius*.

M. minima, Marotel 1912

M. triangularis, Marotel 1913

M. conjungens, Sauter 1917

M. latifrons, Sauter 1917

M. crassicollis, Sauter 1917

M. parva, Sauter 1917

M. pellucida, Blei 1920

M. translucida, Jenkins 1923

M. chappuisi, Baer 1923

The question of the validity of these species will be considered below.

GENERAL STRUCTURE OF THE GENUS *MONIEZIA*

The segments are usually very much broader than long (fig. 9), though worms may be met with in which the breadth may only be three times the length (figs. 6 and 7). These variations are attributable to the state of contraction or relaxation of the different sets of muscles, and to a certain degree they are specific. Thus, as a rule, *M. planissima* has a broader strobila than *M. expansa*. The actual shape of the segment, or the length of the complete strobila, cannot be considered as of specific value. I have been able to stretch a fragment of live worm to at least five times its original length, without damaging it. Also most worms undergo an appreciable amount of contraction when placed in weak formalin, and further contraction may take place when clearing in clove oil.

The length of the strobila also depends on the age of the worm; for instance, an individual which has but newly established itself will be short, and mature segments will be reached within a short

distance of the scolex. I have seen specimens of the *expansa* group, 30-50 cms. in length, in which the last segments were gravid. In older worms the strobila may be anything up to three metres long. In this case the difference between any two succeeding proglottides is not appreciable, and maturity will only be arrived at at a considerable distance from the head. I have seen several fragments, measuring 70 cms.-1 metre in length, in which all the proglottides were at the same stage of development.

The amount of overlapping is also dependent on the state of contraction of the various muscles. One individual may show broad proglottides with overlapping edges, where the longitudinal muscles have contracted, whereas a few inches further back the longitudinal muscles may be relaxed and the circular muscle-layer be contracted, giving the segment a longer, narrower appearance, with hardly any overlapping of the posterior edges.

The question of shape and size has here been gone into in detail, because these points have been taken to be of specific value.

All the authors are agreed that the shape of the head and the length of the neck are liable to show a great deal of variation. On the whole, the scolex and neck of the *expansa* group can be said to be long and slender, whereas in the *planissima* group they are squat and broad, even when in a fairly relaxed state. I have drawn several heads (figs. 1-5), all taken from typical *Moniezia expansa*, showing the shapes they may assume, according to the sets of muscles contracted. Fig. 3 resembles the head of *M. expansa* as drawn by Stiles and Hassall (1893), whereas fig. 5 corresponds with their drawing of *M. oblongiceps*.

The nervous, excretory and muscular systems are essentially the same throughout the genus (see Tower 1900, Zschokke 1888) so need not be dealt with here.

The Expansa Group

In this group the cells of the interproglottidal glands are arranged around blind sacs. To this group have been ascribed *M. expansa*, *M. oblongiceps*, *M. trigonophora*, *M. minima*.

The interproglottidal glands are usually well-developed and stain readily. In material, however, which had been preserved for several years, I occasionally had great difficulty in making them take the stain at all. They were likewise often very difficult to see

FIG. 1.

FIG. 3.

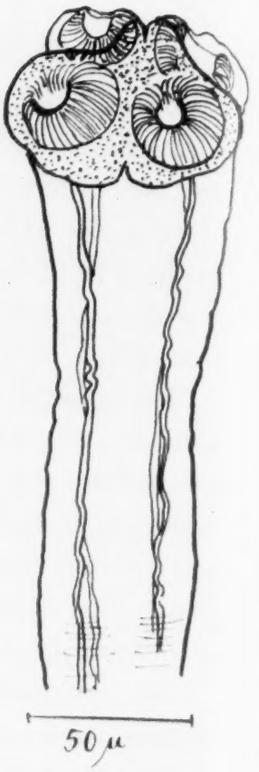

FIG. 4.

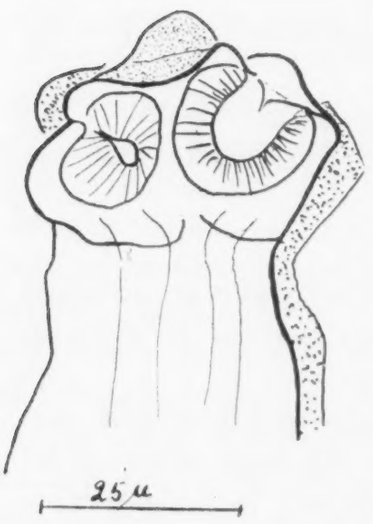

FIG. 2.

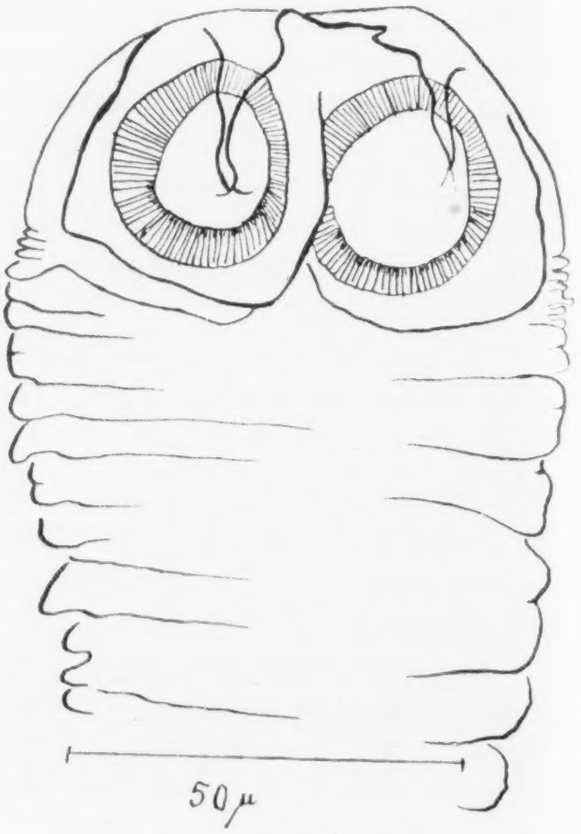

FIG. 5.

FIGS. 1-5. Heads of typical specimens of *M. expansa*.

in strongly-contracted specimens. Nevertheless, a diligent search of the whole strobila never failed to reveal their presence. In relaxed specimens, where the glands were easily visible, they were seen to increase in number, in proportion to the width of the segment. In gravid segments they may be obscured by the number of eggs present (fig. 12). Among the numerous specimens examined, I found one worm, apparently normal in all respects, and in a relaxed condition, which showed some of its segments devoid of all traces of glands. After every seven or eight ordinary segments, one or two of these abnormal ones were interposed.

Stiles and Hassall give the smallest number of glands as twenty-five for *M. expansa*. I have seen many specimens of typical *M. expansa*, collected in this country, in which the number was much less. As few as seven glands may often be met with, and some mature segments may never have more than about fifteen. Thus the number of glands present and their prominence cannot be taken as of specific value.

The arrangement of the female genitalia is essentially the same in all the species described.

The arrangement of the testes, however, shows a certain amount of variation. In *M. trigonophora*, the testes are roughly arranged in two right-angled triangles; the base of the triangle is nearly parallel to the posterior edge of the segment; the perpendicular is parallel with the lateral margin; the hypothenuse runs from the antero-lateral portion to the posterior edge near the median line. These triangles never meet in the mid-line. In *M. expansa*, according to Stiles and Hassall, numerous testes are present, and they occupy the median line as well as the rest of the median field. According to Zschokke, the testes of *T. expansa* are arranged in two triangles, one each side of the segment, as is the case in *M. trigonophora*. Stiles and Hassall further remark that by far the greater number of segments of *M. expansa* examined by them did not present this relation, although in a few segments of this species, which were collected at Paris, the triangular arrangement of the testes is very distinct. In Rudolphi's specimens the testes are not visible.

My experience is that the testes in *M. expansa* may be roughly arranged in two broad triangles which usually meet in the mid-line (fig. 6), or in a continuous band thinning slightly towards the

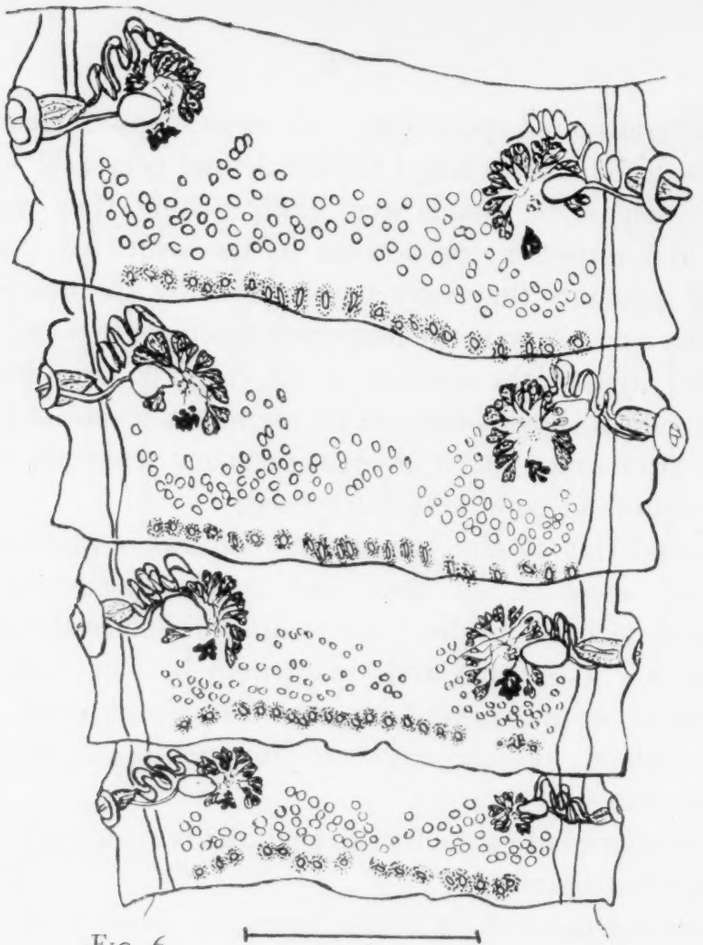

FIG. 6.

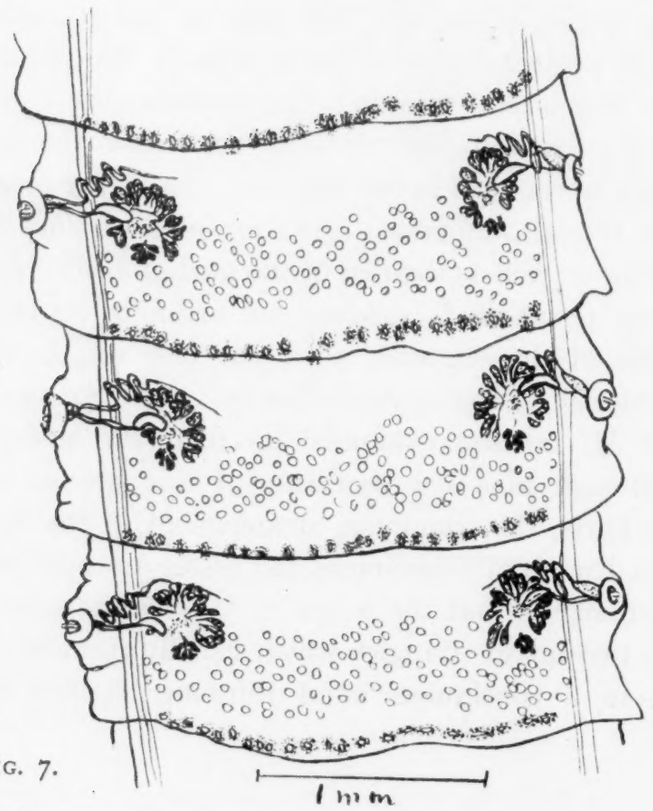

FIG. 7.

FIGS. 6 and 7. Segments of *M. expansa* showing irregular arrangement of testicular fields.

mid-line (fig. 7), or they may be as numerous in the median field as elsewhere (fig. 8). All three types may be found in one individual, or only one type may be present. On the whole, I am inclined to think that this question of arrangement also depends on the state of contraction of the segment. It can be readily conceived that Fig. 7 is Fig. 6 with the circular muscles slightly contracted, and that in Fig. 8 we have still further contraction of the circular muscles. As Child rightly points out, the arrangement of the reproductive organs may vary widely in correlation with the variation in form of the proglottid. There is also a variation in the number of testes in different segments of one strobila.

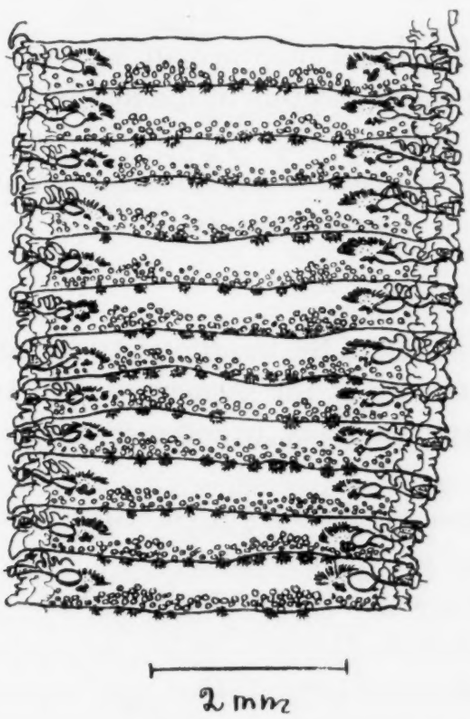

FIG. 8. Contracted segments of *M. expansa*, showing testes arranged in a continuous band.

M. oblongiceps agrees with *M. expansa* in all respects, except that the testes are smaller and less numerous. The description of this species is based on two entire strobila and one fragment. As both specimens are under one metre long and are gravid, it seems that we are dealing with a parasite newly established in the gut. The description, when regarded from this point of view, agrees with that of a young *M. expansa*. Thus *M. oblongiceps* must be considered as a synonym of *M. expansa*.

The characteristic features of *M. minima* are given as follows : gravid segments square (anneaux ovigères carrés) ; testes arranged in a continuous band. As pointed out above, the shape of the segments depends entirely on the amount of contraction present (figs. 6-8), and the arrangement of the testes shows a considerable amount of variation. Hence *M. minima* must be regarded as a variation of *M. expansa*.

The *expansa* group thus contains but two species : *M. expansa*, in which the testes are roughly arranged in a band stretching to the excretory vessels on either side, and *M. trigonophora*, in which the testes are arranged in two triangular fields that do not meet in the mid-line.

It is possible that forms intermediate between *M. trigonophora*, with its two triangles not meeting in the mid-line, and the variation of *M. expansa* as drawn in Fig. 6, may still be found. However, until further variations have been demonstrated, it is preferable to consider the two as separate species.

The Planissima Group

In this group the cells of the interproglottidal glands are arranged in a line at the juncture of the segments. Ten species have been described which have this linear arrangement of the interproglottidal glands, namely :—

M. benedeni (Moniez 1879), R. Blanchard 1891
M. neumanni, Moniez 1891
M. planissima, Stiles and Hassall 1892
M. triangularis, Marotel 1913
M. conjungens, Sauter 1917
M. latifrons, Sauter 1917
M. crassicollis, Sauter 1917
M. parva, Sauter 1917
M. pellucida, Blei 1920
M. translucida, Jenkins 1923

The glands in this group have been described as short and ill-defined (*M. triangularis*), or large and distinct (*M. planissima*), or first linear and later arranged round three blind sacs (*M. conjungens*). These differences are more apparent than real, as the glands in any one strobila may vary a great deal in length. The dorsal gland may

be considerably longer than the ventral, or *vice versa*, or both may be approximately equal in length (fig. 9). Again, the linear gland may be broken up into smaller parts (fig. 9). I have never observed these cut-off portions to be arranged around blind sacs as described

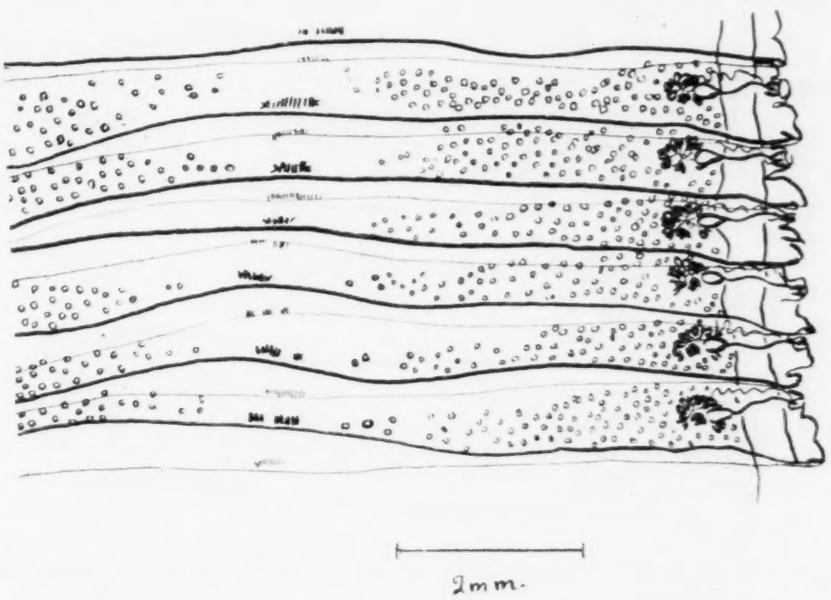

FIG. 9. Segments of *M. planissima*, showing testes arranged in two triangles; glands entire or broken.

by Sauter; on the contrary, the glandular cells always persist in their linear arrangement. In all the relaxed specimens examined, glands were discernible in each proglottid.

The testes in this group, as in the *expansa* group, show a great deal of variation in their arrangement. In the usual typical form they are arranged in two irregular triangles which may or may not meet in the mid-line (figs. 10 and 11), the two triangles may be some distance apart (fig. 9), or the testes may merge into one another and form a continuous band, stretching from the one longitudinal excretory vessel to the other (fig. 12). These differences may appear indiscriminately in young immature segments, in old segments, or in adjoining segments.

I could establish no constant relationship between the average width of the segment, the arrangement of the testes and the length of the gland or its tendency to break up into smaller portions. On the contrary, all the evidence goes to prove that each of these factors varied independently of the others. The different combina-

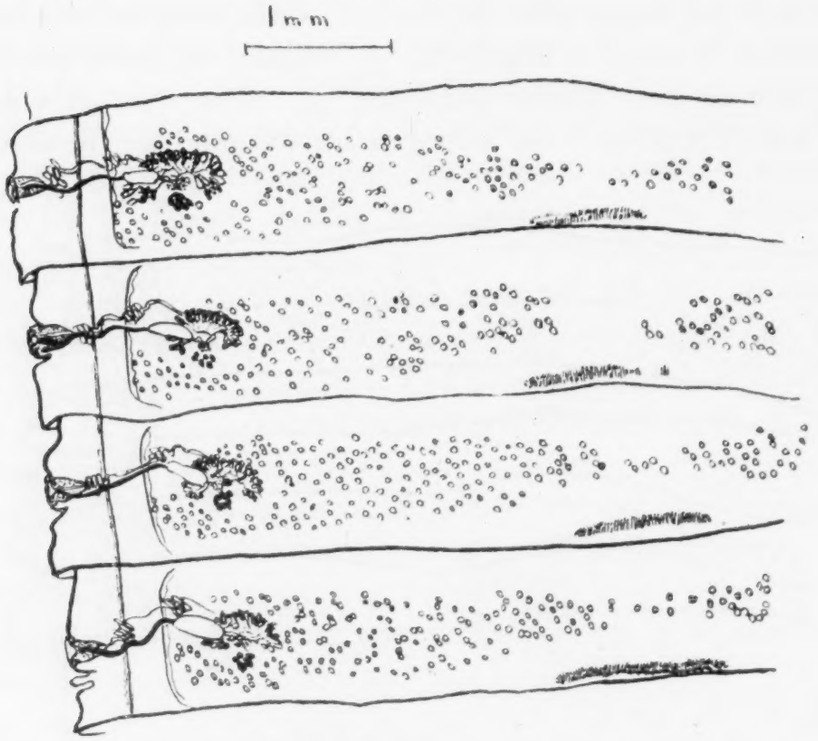

FIG. 10. *M. planissima*, showing irregular arrangement of testicular fields; glands entire or broken.

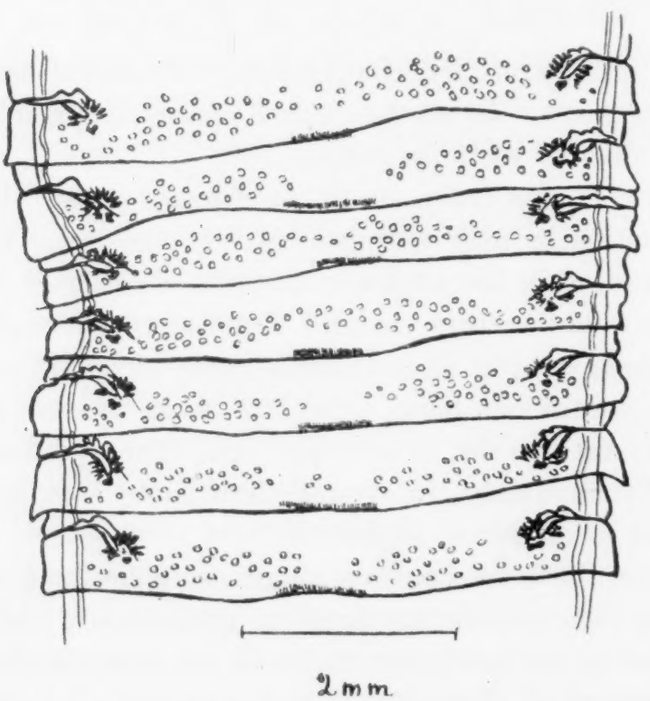

FIG. 11. *M. planissima*, showing irregular arrangement of testicular fields. In this strobila this irregular arrangement persists until maturity is reached. In later segments the glands are broken up.

tions possible would give the various species described; as, for example: small linear gland, testes in two triangles = *M. triangularis*; small linear gland, testes in a continuous band = *M. neumanni*; large linear gland, testes sometimes in two triangles and sometimes a continuous band = *M. planissima*; large gland, testes in a continuous band = *M. translucida*. Unfortunately, however, these arrangements cannot be considered as constituting separate species, as any number of intermediate forms are possible; they are rather to be looked upon as variations of *M. planissima*, with the other described species as links in the chain. In his account of

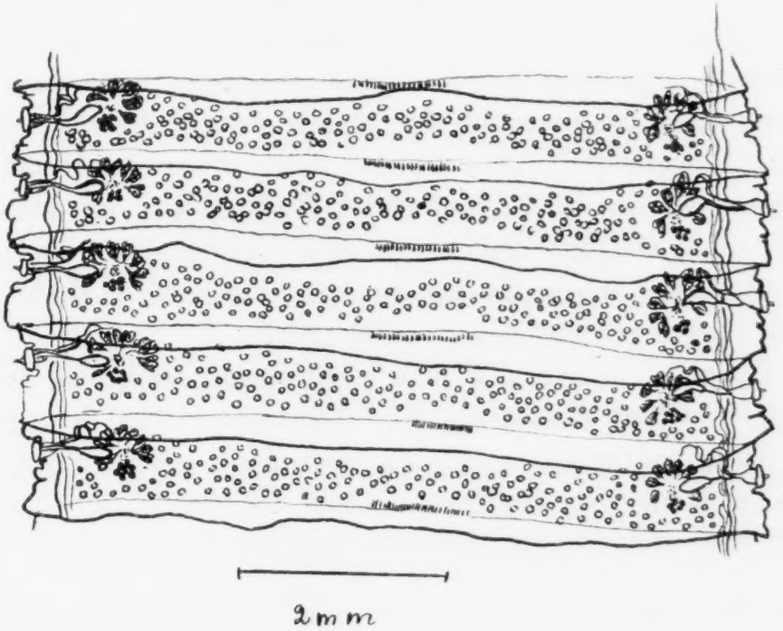

FIG. 12. *M. planissima*, showing testes arranged in a continuous band; glands entire or broken.

M. conjungens, Sauter (1917) describes the interproglottidal gland as first linear and later arranged around three blind sacs. As I have never seen this arrangement, but as the linear gland is often broken up into several smaller parts, I assume that this is what Sauter saw, although he draws them arranged round blind sacs.

The *planissima* group thus contains but one species, which will have to be designated *M. benedeni*, in which the head is comparatively large, the testes may be arranged in two triangular fields, or in a continuous band; and the proglottidal gland is linear, and of varying length.

The Alba Group

This group is based on the fact that its members have no interproglottidal gland. In the material put at my disposal by Mr. Southwell were several bottles labelled *M. alba*, the determination in each case having been based on the examination of a fragment taken from about the middle of the worm. On examination of the whole strobila, however, interproglottidal glands were invariably found under high magnifications. Most of these specimens were either much contracted and distorted, or had been kept in alcohol for a considerable length of time, so that they did not stain well.

With only the above evidence to support the theory, it would be unjustifiable to deny that there may be *Moniezia* without any glands at all, especially in view of the fact that I have seen a well-preserved specimen in which the glands were definitely absent in some of the segments. This absence of the interproglottidal glands may either be due to degeneration of the worm, or possibly to maceration of the material, or to some other cause. Until more work has been done on the presence and function of these glands, *M. alba* must be accepted as a valid species.

Baer (1923) described a new species, *M. chappuisi*, from an antelope, in which no glands were discernible. His description is based on a contracted specimen, and as it shows no essential differences from the description given for *M. alba*, it must be considered as synonymous with that species.

Species Inquirendae

Moniezia rugosa (Diesing 1850), Lühe 1895

Lühe 1895 re-examined Diesing's original specimen of *T. rugosa* from the monkey, *Ateles hypoxanthus*. He describes it as a typical *Moniezia*, but does not mention any glands. His oldest segments were not mature enough to enable him to describe the eggs.

If this is a true *Moniezia*, it will fall into the *Alba* group. The question of its validity can only be settled by further work on the parasites of *Ateles*.

Moniezia amphibia, v. Linstow 1901

This worm is reported from *Hippopotamus amphibius*, where it was present in large numbers. From the description and figures

given, it is difficult to decide whether it is a *Moniezia* without interproglottidal glands, or any other double-pored anoplocephalid. In the drawing of the egg, the horns of the pyriform apparatus may be interpreted as possessing a disc or as having their tips bent over.

Until the original material has been re-examined, this species will have to be considered as a *Moniezia*, of the *Alba* group.

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TRYPANOSOMA EVANSI AND *ORNITHODORUS CROSSI*

BY

WARRINGTON YORKE

AND

J. W. S. MACFIE

(*Received for publication* 25 *March*, 1924)

A series of experiments recently published by Cross (1921-23) suggests that *Trypanosoma evansi* of the camel in India is biologically transmitted by the tick *Ornithodorus crossi*, Brumpt, 1921. Cross fed *Ornithodorus crossi* on dogs heavily infected with *T. evansi* and found that when the ticks were, after various intervals, subsequently fed on clean animals, some of the latter became infected. In his earlier paper (1922) he states that ' These ticks transmitted the disease to healthy rabbits sixty-seven, eighty-three and one hundred and one days after feeding on an infected-surra animal, but they were not infective after one minute to forty-six days. This would apparently show that there is a life cycle of the trypanosome within the tick ' ; and in a later paper (1923) that ' These ticks were found capable of spreading the disease to a healthy animal seventeen days and one month after feeding on an infected animal.'

On 10 January, 1923, we received from Captain Cross about two hundred *Ornithodorus crossi*, which had been on 4 December, 1922, fed on a dog with numerous *T. evansi* in its blood. These ticks were divided into four batches, the first three being fed on rabbits and the fourth on a guinea-pig. Between January and June, 1922, the ticks were given frequent opportunities of feeding, but none of the rabbits nor the guinea-pig became infected. It should be mentioned that from the time of their arrival in England, viz., 10 January, 1923, the ticks were kept in an incubator at about 20° C.

It occurred to us, however, that a possible explanation of the failure of these ticks to infect was the fact that immediately after

the infecting feed they were subject to relatively low temperatures during their journey from India to England in the month of December. In order to examine this question Captain Cross sent us a second supply of about two hundred *Ornithodorus crossi*, which were fed on a heavily infected dog on 23 July, 1923. These ticks, which were received by us on 24 August, 1923, were divided into two batches, each of which was allowed to feed on a rabbit on 24 August, and at frequent intervals subsequently up to date: no infection resulted.

We have no explanation to offer for these negative results, but the subject is one of such considerable practical importance as to demand re-investigation.

In order to ascertain what happens to trypanosomes taken into the stomach of the ticks, we fed on 27 June, 1923, a number of the first lot sent by Captain Cross on a rabbit in the blood of which were numerous *T. rhodesiense*. Two months later, two of these ticks were dissected and were found to contain blood in which large numbers of motionless trypanosomes were not only easily recognisable, but were in a state of remarkable preservation. A suspension in normal saline solution of the stomach contents of one of these ticks was inoculated into a mouse intraperitoneally, but failed to produce an infection. In a second, similar, experiment, a tick was dissected on the day after it had fed on a rabbit infected with *T. rhodesiense*. The trypanosomes found in this tick were also motionless and appeared to be dead, and when inoculated intraperitoneally into a mouse failed to infect it.

This observation does not, of course, touch the question of the possible biological development of *T. evansi* in the ticks, but is of interest in showing that trypanosomes which die rapidly in the stomach of the tick remain there for prolonged periods in an excellent state of preservation.

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MISCELLANEA

DURATION OF INFECTION IN MALARIA

(*a*) *Quartan.*

P. left India, 1910. No fever and no quinine taken while in England, until November, 1915, when quartan parasites were found in the blood.

(*b*) *Simple tertian.*

N. 9.10.1921, simple tertian parasites.

February, 1922, left West Indies where it is possible a new infection was contracted.

February, 1922, to January, 1924. Sailing between England and North America only.

21.1.24 simple tertian parasites.

J. W. W. STEPHENS.

STRONGYLIDAE FROM EDINBURGH HORSES

Since the beginning of October I have had the opportunity to collect and partly examine the Strongylidae found in horses in the Scottish capital. As some of the species present have not as yet been recorded as occurring in either England or Scotland, I thought it interesting enough merely to give a list of the species I have been able to identify so far, but hope to deal more fully with this subject at a later date.

The following are present in the collected material :—*Strongylus vulgaris, S. equinus, S. edentatus, Gyalocephalus capitatis, *G. equi, *Craterostomum mucronatum, Trichonema coronatum, T. bicoronatum, T. calicatum, T. poculatum, T. insigne, T. goldi, T. euproctus, T. nassatum, *T. nassatum* var. parvum, **T. tridentatum, T. mettami, *T. minutum, *T. longibursatum, *T. pseudo-catinatum, *T. pateratum, *T. labratum, *T. labiatum, *T. radiatum.*

P. L. LE ROUX, B.Sc., M.R.C.V.S.

Zoology Department,
University of Edinburgh,
17.12.23.

* To my knowledge these nematodes have not as yet been reported to occur in British horses, except a foal which furnished Yorke and Macfie with examples of *Gyalocephalus equi* and *Trichonema pseudo-catinatum* after it had been in the same field as American horses which died shortly after importation in 1918.

ANCYLOSTOMES IN A LEOPARD

Six specimens of *A. braziliense*, Gomez da Faria, 1910, three males and three females, were recovered from a young leopard in Sierra Leone. The females varied from 7·7 to 9·4 mm. in size, the males from 6 to 7 mm.

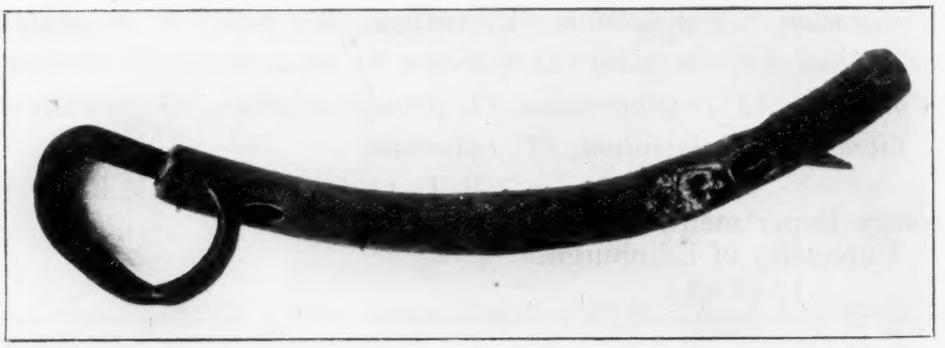
Eleven specimens of *A. caninum* (Erc., 1859) Hall, 1913, were also recovered, six males and five females. The females varied in size from 7·3 to 8·5 mm., the males from 5·6 to 7·5 mm.

S. ADLER.

OCCURRENCE OF *ASCARIS LUMBRICOIDES* IN A DRAINAGE TUBE

Dr. Webb Anderson of China has presented to the Liverpool School of Tropical Medicine a specimen of *Ascaris lumbricoides* found in a drainage tube from an appendix abscess. The patient, a Chinese woman, was operated on successfully for appendicitis. Progress was normal for three or four days, after which drainage ceased, and was accompanied by a rise of temperature. On removing the drainage tube it was found that a specimen of *A. lumbricoides* had entered the tube, thus preventing drainage. A photograph of the worm *in situ* is shown below.

T. SOUTHWELL.



Natural size — *M. Brown*

OPHIOTAENIA MARENZELLERI

A specimen of *Ophiotaenia marenzelleri*, La Rue 1911, (= *Ichthyotaenia marenzelleri*, Barrois 1898) was found in the intestine of *Causus rhombeatus* in Freetown, Sierra Leone.

This cestode has previously been recorded by Barrois from *Ancistrodon piscivorous* in the southern United States of America.

T. SOUTHWELL and S. ADLER.

ZSCHOKKEELLA GUINEENSIS

Zschokkeella guineensis (Graham, 1908) was found in the intestine of a ground pig, *Thrinomys swinderianus*, in Freetown, Sierra Leone.

This parasite has previously been recorded from *Cricetomys gambianum* in Accra.

T. SOUTHWELL and S. ADLER.

TETRARHYNCID LARVA

A Tetrarhyncid larva was found in the liver of a fish *Diodon hystrix* caught near Freetown, Sierra Leone.

T. SOUTHWELL and S. ADLER.

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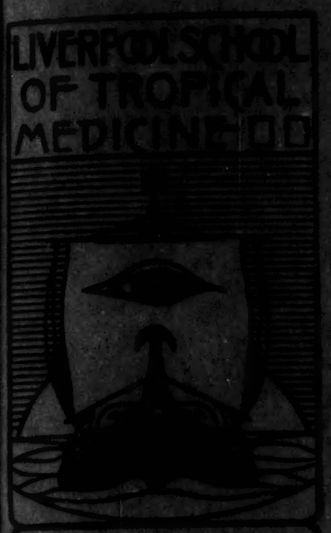

ANNALS

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P. 34. For Plate II *read* Plate III.
For Plate III *read* Plate II.
P. 35. Line 6, delete the words Fig. 13.
P. 114. For 50μ and 25μ *read* 500μ and 250μ.
P. 129. For Tetrarhyncid *read* Tetrarhynchid.

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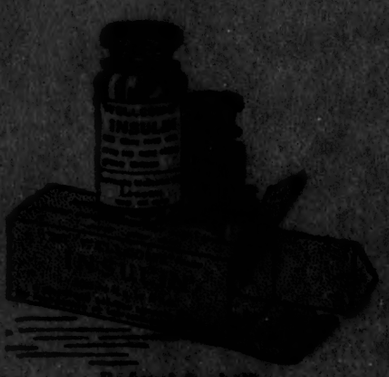

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A NOTE ON *PLASMODIUM AGAMAE* (WENYON, 1908)

BY

S. ADLER.

(*Sir Alfred Lewis Jones Research Laboratory, Freetown, Sierra Leone*)

(*Received for publication*, 28 *February*, 1924)

Wenyon (1908) working in the Sudan, described a protozoon from the red cells of a lizard *Agama colonorum*, which had the following characters :

The smallest forms found in the red cells were oval or pear-shaped and contained no pigment, but larger immature forms containing brown pigment were also found ; the sexual forms, microgametocytes and macrogametocytes, were sausage-shaped. The affected red cells were not altered in shape or size, but the nucleus was in some instances slightly displaced by the sexual forms of the parasites.

Schizonts were very few and only the early stages were found in the blood ; in no case was a schizont found which had segmented into merozoites. Wenyon, therefore, assumed that multiplication took place in the internal organs after the manner of asexual multiplication, as described by Arago for *Halteridium* and he concluded that the parasite he found in *Agama colonorum* had affinities to *Halteridium* and suggested the name *Haemoproteus agamae*, Wenyon (1908).

It must be pointed out, however, that the animal from which Wenyon described his parasite was not heavily infected.

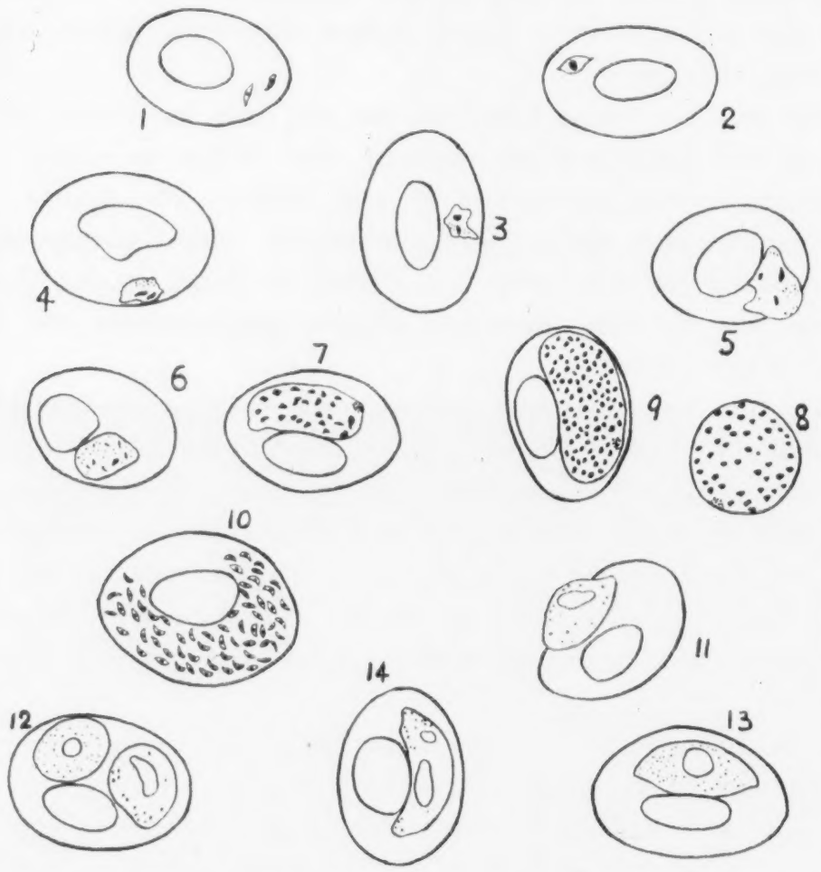
Todd and Wolbach (1912) reported and figured a pigmented haemocytozoon from *Agama colonorum*, which closely resembled the parasite described by Wenyon, but the small forms of the parasite were not found.

Macfie (1914) working in Nigeria, found in the blood of three lizards pigmented parasites which he considered to belong to the same species as those described by Wenyon.

The author found in Sierra Leone a specimen of *Agama colonorum* heavily infected with a pigmented haemocytozoon which appears to be the same species as that described by Wenyon under the name *Haemoproteus agamae.*

Asexual forms, trophozoites and schizonts, were numerous, and it was found that schizogony takes place in the red cells in the peripheral blood. The parasite, therefore, has no affinites to *Halteridium*, but is in fact, a *Plasmodium* and its name should be *Plasmodium agamae* (Wenyon, 1908).

The parasite was studied in smears, and the following points were noted. The smallest forms found in red cells are minute, and



FIGS. 1 and 2. Young trophozoites in a red cell.
FIGS. 3, 4 and 5. Amoeboid forms with several masses of chromatin.
FIGS. 6—9. Further stages of schizogony.
FIG. 10. Schizont which has segmented into merozoites.
FIG. 11. Young microgametocyte, partly outside the red cell.
FIG. 12. A red cell containing a young microgametocyte and a young macrogametocyte.
FIG. 13. A macrogametocyte.
FIG. 14. A microgametocyte. × 1200.

spindle-shaped, and contain no pigment (figs. 1 and 2). Slightly larger forms are found, very irregular in shape, containing fine granules of pigment and two or more nuclear masses (figs. 3 to 5). Such forms are commonly found in the red cells, but they may also occur either free or partially in the red cells. It seems, therefore, that trophozoites and young schizonts can travel from one red cell to another, unless the free or partially free forms are due to an artifact. Multiple infection of the red cell is common. As the schizont increases in size the nucleus of the red cell is displaced.

The pigment tends to be extruded from the schizont during its growth (fig. 7), and the mature schizont may contain no pigment at all; the mature schizont contains about seventy merozoites.

The young sexual forms are round or ovoid and are found both free and in the red cells. A few instances were noted in which a young sexual form was found partly inside and partly outside a red cell (fig. 11).

The mature sexual forms are crescentic in shape and seldom displace the nucleus of the red cell (figs. 13-14).

The macrogametocyte stains deep blue with Giemsa, its pigment granules are very fine and are uniformly scattered throughout the protoplasm. The microgametocyte stains pale blue with Giemsa, its pigment is coarser than that of the macrogametocyte and tends to be distributed round the rim of the parasite. The protoplasm of the microgametocyte was in several instances observed to be vacuolated (fig. 14).

RAT-FLEAS IN FREETOWN, SIERRA LEONE

BY

B. BLACKLOCK

AND

M. G. THOMPSON

(From Sir Alfred Lewis Jones Research Laboratory, Freetown)

(Received for publication 30 March, 1924)

Hirst's (1923) work has attracted attention to the importance which must be attached to the relative proportion of various species of *Xenopsylla* on rats in places which appear liable to plague epidemics. He concluded from his observations that *X. astia* was a much less efficient transmitter of plague than is *X. cheopis*.

In Freetown a hundred rats sent by the Sanitary Department were examined during the months of January and February, 1923: the rats came from various parts of the town; the numbers were:—

Black rats	62
Brown rats	38

All the fleas removed from the rats were collected, a total number of 657. Of these 654 belonged to the genus *Xenopsylla* and 3 to the genus *Ctenocephalus*. The 654 *Xenopsylla* comprised 419 fleas of the species *X. brasiliensis* Baker (1904), and 235 of *X. cheopis* Rothschild (1903). The *Ctenocephali* belonged to the species *C. canis* Dugès. In the table are shown the numbers and sex of the rats harbouring *Xenopsylla* and the species recovered from them.

No *X. astia* were found on these rats; Evans (1922), however, records this species among rat fleas sent to her from the Gold Coast.

Xenopsylla brasiliensis, Baker (1904) was originally described from Sierra Leone, but its capacity for transmitting plague bacilli has not

so far been worked out. Whether it exhibits that relative inability which Hirst attributes to *X. astia* in Ceylon is unknown.

It is of interest to note that Newstead and Evans (1921), who examined 469 black rats from ships in Liverpool (59 of these rats being obtained from ships coming from various West African Ports), do not record any *X. brasiliensis* from the 469 rats examined, whereas they found 489 *X. cheopis*. Again, Balfour (1922) did not report any *X. brasiliensis* on 34 black and 444 brown rats obtained mostly in London, whereas he records *X. cheopis* on 5·9 per cent. of the black rats and 3·6 per cent. of the brown rats. Hirst (1923) states that *X. brasiliensis* is found on the rats of West Africa, South America and the uplands of Peninsula India.

TABLE I.

	Total	Number infested	*X. brasiliensis*	*X. cheopis*
Black Rats ♂	21	19	68	36
,, ♀	41	30	74	75
Brown Rats ♂	17	15	185	87
,, ♀	21	19	92	37
	—	—	419	235

Cragg (1920) states that *X. brasiliensis* is not common in India and cites Poona, Mangalore, Bombay City, and Ootacamund as the only places from which it had then been received by him.

Owing to the smallness of the numbers of West African ship-rats examined here and in Liverpool, it is not yet possible to say whether *X. brasiliensis* is capable of remaining alive during transport on ships to England.

Whether *X. brasiliensis* is a plague-transmitting flea or not, it appears probable from our figures that there is a sufficiently large percentage of *X. cheopis* present on rats in Freetown to carry plague effectually in epidemic form, should this disease be imported.

The number of rats in Freetown is large, and, owing to the extensive area which is at present capable of providing natural

shelter for them, it is evident that vast numbers would survive any ordinary efforts at reduction.

The city of Freetown possesses many quarters in which the native population is overcrowded and it contains, also, a large floating population. All these factors are of importance in the case of plague epidemics. The chief protection of this port, in the past, against the introduction of plague from other coastal regions has probably been the absence of a deep-water wharf.

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A CONTRIBUTION TO THE KNOWLEDGE OF THE TREMATODE PARASITES OF THE FOOD MAMMALS OF RANGOON

BY

G. D. BHALERAO, M.Sc.

(Biological Department, University of Rangoon)

(Received for publication 4 April, 1924)

PLATES VI-VIII

TABLE OF CONTENTS

INTRODUCTION

The present paper is the outcome of an examination of the Trematodes found in the bullocks, cows, buffaloes, goats, sheep, and pigs slaughtered at the municipal abattoirs, Rangoon. *Fasciola gigantica*, Cobbold, 1858, was found only in bullocks, cows, and buffaloes, always in the liver, and in large numbers. Thus the infection in buffaloes is sometimes 10 per cent., and in bullocks and cows 4 per cent. and 2 per cent. respectively. *Paramphistomum cervi* (Zeder, 1790) and *Eurytrema dajii*, n.sp. sometimes accompany *F. gigantica* in the bile ducts of *Bos indicus*. In goats and sheep the infection is very small, amounting in some months to only 2 per cent. This infection, as reported by the municipal officials, is due to *Fasciola hepatica* L. 1758. No opportunity presented itself of examining these parasites, all the goats and sheep proving to be non-infected whenever a visit was paid to the slaughter-house. Two varieties of pigs are killed in Rangoon, one from Burma, the

other imported from the east coast of India, the latter of these being specially rich in Trematodes. Two species occur in their intestines, *Fasciolopsis fülleborнii*, Rodenwaldt, 1909, and *Testifrondosa cristata*, n.sp., the latter being rare.

It is my pleasant duty here to thank Professor F. J. Meggitt who helped me at almost every stage of my work and Mr. G. E. Gates of Judson College, who kindly allowed me the use of his preparations of *Testifrondosa cristata*.

PARAMPHISTOMUM CERVI (Zeda, 1790)

Numerous specimens of this species were found in the bile-ducts of *Bos indicus*, together with many *Fasciola gigantica*. The material confirmed in all respects the description already given by Maplestone (1923).

FASCIOLA GIGANTICA, Cobbold, 1858

Innumerable specimens of this species agreeing in all points with the ' Rangoon specimens ' of Jackson (1921) were found blocking the bile-ducts of *Bos indicus* and *Bos bubalis*. *F. aegyptica*, Looss 1896, appears to be identical with it.

F. HEPATICA, L. 1758

Specimens of this species are reported to have been found in the bile-ducts of sheep and goats, but no cases have been observed by me and no definite records exist.

FASCIOLOPSIS FÜLLEBORNII, Rodenwaldt, 1909 (Plate VI)

Many specimens of this species were found in the intestines of pigs (*Sus cristatus*, Wag., 1900) killed at the slaughter-house, Kemmendine, Rangoon. Apparently they are not firmly attached to the walls of the intestine, as numbers of them quickly emerge when it is washed. The specimens ranged from very immature forms (5·5 mm. by 2·2 mm.) to large ones (55 mm. by 16 mm.), both measured living and fully extended in luke-warm water. These specimens undergo very great contraction when placed in a fixing fluid and become very thick. The immature forms are

rather elliptical, with their greatest width at the level of the anterior testis and with no indication of a cephalic cone at their anterior ends.

The mature forms appear to be tongue-shaped when completely extended and at their anterior end a short cephalic cone can be made out, the posterior end being bluntly rounded. The greatest width is attained at the level of the anterior testis. The cuticle is thick and smooth without either spines or scales.

At the end of the cephalic cone in the fully developed forms is a small circular mouth, surrounded by an oral sucker also circular and measuring from 270μ to 700μ in diameter. At a distance of almost 300μ behind the oral sucker is a large ventral sucker (700μ in diameter and from 1·54 to 2·8 mm. in length) produced posteriorly into a sac-like prolongation. The ratio between the oral and the ventral sucker is 1 : 3·2 to 1 : 3·4. Immediately following upon the oral sucker is a prepharyngeal sphincter behind which is a globular pharynx 240μ to 670μ in diameter. There is no oesophagus. The intestinal caeca appear to arise from the pharynx and pass posteriorly almost to the hinder end of the body in a zigzag manner with two characteristic curves, one in front of the anterior testis between it and the ovary and another between the testes. The thickness is uniform throughout.

The genital pore is immediately anterior to the ventral sucker. The cirrus sac is very characteristic. It is not a cylindrical straight pouch as in other species, but is peculiarly convoluted. It extends from between the shell gland and ventral sucker and passes anteriorly, touching the right border of the latter organ. In a well-grown specimen its width is 700μ and its length 8·5 mm. The posterior end is at the posterior two-thirds or more of the distance between the shell gland and the hinder border of the ventral sucker, the exact distance depending on its degree of convolution which is more pronounced in adults. In immature forms where the sac is nearly a cylindrical tube, its posterior end almost approaches the shell gland. The testes are the most prominent organs in the body, occupying nearly half its total area, lying one behind the other, and extending posteriorly from the shell gland. They are separated from the posterior border and the sides by the vitellaria. They are very much branched, each with four main branches, two diverging anteriorly and two posteriorly from a central point. In a fully

grown and well extended specimen the anterior testis measures 9 mm. antero-posteriorly and 12·5 mm. laterally, the posterior, 8·5 mm. antero-posteriorly and 12 mm. laterally. In no case, mature or immature, was the anterior testis found to be smaller than the posterior as Rodenwaldt (1909) has stated. The vas efferens from the posterior testis arises from the centre and passes anteriorly under the anterior testis, after which it turns towards the right to pass on the same side of the shell gland. In front of this it curves towards the centre, to meet a little behind the cirrus sac with the corresponding vas efferens from the anterior testis coming from the left side of the shell gland. The vas deferens so formed continues anteriorly for a short distance, then enters the cirrus sac, inside which it immediately swells into a seminal vesicle. This is continued anteriorly in a peculiarly convoluted course to the middle of the ventral sucker, where it is surrounded by the pars prostatica, then as the cirrus, finally opens by the male pore into the genital sinus.

The ovary is small and very much branched, measuring 230μ by 190μ in immature specimens and 2·85 mm. by 1·6 mm. in the fully mature forms. It lies to the right side of the body in front of the anterior testis, between it and the uterine coils. This position is liable to very interesting variations. In immature forms the ovary lies almost exactly in the middle of the body, but as the posterior half increases in size faster than the anterior, its centre moves more and more anteriorly as growth proceeds, until at last, in the fully developed specimens, it comes to lie in the anterior two-fifths of the body. From its inner side a short oviduct passes into the shell gland which, in the immature specimens, is somewhat oval measuring 240μ by 290μ, and in the mature forms almond-shaped, measuring 2·5 mm. by 1·55 mm. It lies in the centre in front of the anterior testis, always in the same position with regard to the ovary. On the ventral side is a spherical receptaculum seminis, measuring in fully developed specimens 650μ in diameter. In the immature forms no such organ is developed. Laurer's canal is short and opens on the dorsal side. The uterus becomes visible at the shell gland. From thence it passes to the left and again to the right, thus describing several loops between the shell gland and the ventral sucker. As the vagina, it is continued anteriorly by the side of the cirrus sac and dorsally to the ventral sucker to open into the genital sinus

through the female pore to the left of the male aperture. In some of the sectioned specimens, the greater part of the uterus was observed to be filled with spermatozoa. The vitelline glands are well developed and occupy a large area. They extend as lateral bands from the ventral sucker to the posterior end of the body, where both unite in the middle line behind the posterior testis. They consist of numerous round follicles each composed of several small acini. The stout transverse vitelline ducts (140μ in thickness) pass in front of the anterior testis and join in the middle line to form a common duct which opens into a yolk reservoir (1·78 mm. by 1·17 mm.) dorsal to the shell gland. The eggs are oval, thin-shelled and measure from 140μ to 162μ in length, and from 85μ to 95μ in breadth.

The description of the adult forms collected agrees with that given by Rodenwaldt (1909) in all respects, except that the anterior testis is larger than the posterior, whereas in specimens described by Rodenwaldt the contrary was the case. This discrepancy is probably due to the amount of contraction undergone by Rodenwaldt's specimens in the process of fixation. Further, his description was based upon only two specimens. More extensive material would possibly have caused a revision of his results with regard to this point. There are, in addition, slight differences of dimensions, but this point cannot be regarded as important, dependent as it is on the age and state of contraction of the worms.

TESTIFRONDOSA CRISTATA, n.g., n.sp. (Plate VII)

About three dozen specimens were found on one occasion in the intestine of *Sus cristatus*. The body of the worm is flat, with almost parallel sides which taper anteriorly, and it is covered with a thick cuticle. At the posterior end is a notch, above which is the excretory pore. The anterior part of the body from the level of the ventral sucker is thickly covered with backwardly directed scales which become more sparse posteriorly until they disappear completely at the level of the anterior testis. The length of the mature worms varies from 6 to 8 mm., the last being specimens extended under a little pressure. In breadth they vary from 2·5 to 3·5 mm., the greatest breadth being attained at the level of the posterior testis.

The circular mouth at the anterior end is surrounded by an oral sucker, 220μ in diameter. The ventral sucker is much larger than the oral and is drawn out posteriorly into a sac-like prolongation. It is 1·02 mm. long by 0·82 mm. in diameter, the excess of the length over the breadth being due to the sac-like prolongation posteriorly. The ratio between the oral and the ventral sucker is nearly 1 : 4. The usual position of the ventral sucker is with its centre approximately at the anterior fifth of the body.

The prepharynx is small, just behind the oral sucker. No pharyngeal glands were observed. The pharynx is globular with thick muscular walls and a diameter of 180μ. It is followed by an oesophagus 230μ in length. The intestinal caeca are unbranched and reach almost to the posterior end of the body. Each caecum is not uniform throughout, but narrower near the fork and slightly broader posteriorly.

The excretory vesicle is small and pear-shaped and opens behind by a pore on the dorsal side, a little in front of the notch at the posterior end. It extends anteriorly only for a short distance, and into it open several excretory ducts.

The testes lie centrally in the posterior half of the body one behind the other and are enclosed laterally and posteriorly by the vitellaria. They are the most conspicuous of all the organs in the body and occupy little more than one-fourth of its total length. Their most striking peculiarity is their branched appearance, the anterior one being in the form of a horizontal and the posterior approximately in the form of a vertical cross with the anterior limb slightly lateral. All limbs of the cross are branched irregularly. This branching constitutes the distinguishing characteristic of the genus. The dimensions of the anterior testis are 1·43 by 1·13 mm., and those of the posterior 1·31 by 1·18 mm. The vasa efferentia arise from the centre of the anterior end of each testis, and, after a zigzag course, unite to form a vas deferens opening at the base of the seminal vesicle. This latter is an elongated sac lying at the end of the cirrus sac and occupying the last quarter of its length ; it is continued as a convoluted tube filling three-fourths of the length of the cirrus sac and finally opens into a coiled ductus ejaculatorius ending in a short muscular cirrus. No ductus hermaphroditicus was noticed. Surrounding the cirrus and the ductus ejaculatorius, and

opening into them, are the prostatic glands. The cirrus sac is very much elongated and extends from the intestinal fork to some distance behind the ventral sucker. Its anterior fourth, which lies between the intestinal fork and the ventral sucker, is swollen, as is also the posterior fourth behind the ventral sucker. The middle portion is thinner. The position of the cirrus sac varies according to the state of contraction of the body. Normally it lies dorsal to the ventral sucker, but not infrequently it occurs sometimes to the right and sometimes to the left. The genital pore is central immediately behind the intestinal fork.

The ovary is approximately oval. It is situated on the ventral surface a little to the left of the middle line, between the posterior end of the cirrus sac and the anterior testis. The oviduct is small and runs centrally to the large shell gland, situated between the anterior testis and the ovary. Laurer's canal lies horizontally between the shell-gland and the anterior testis. The receptaculum seminis is ventral, almost in the centre of the body at the level of the ovary ; in entire mounts it is obscured by the shell gland. The vitellaria are lateral and numerous, extending from about half the length of the ventral sucker to the end of the body and occupying nearly two-thirds of the entire length. They consist of numerous small round follicles, each composed of a few acini. They are very narrow anteriorly, but become broader and broader posteriorly until, at a little distance behind the posterior testis, they meet in the middle line. Anteriorly they are marginal to the intestinal caeca, but posteriorly they overlap them. Nearly one-fifth of the posterior portion of the body is filled with these glands. Vitelline ducts arise at the level of the shell gland and open in the centre into a yolk reservoir communicating with the oviduct. Beyond this junction the oviduct is continued as the uterus which, describing six or seven turns between the shell gland and the ventral sucker, continues dorsally as a vagina lined with a thick cuticle. It finally opens into the genital pore by the side of the male genital aperture. The eggs are large, oval and operculated, from 110μ to 130μ long by 70μ to 80μ broad.

In the branched nature of the testes, the extent and form of the vitellaria, and the possession of a pouch to the ventral sucker, the present form resembles the genus *Fasciolopsis*, but differs from it

in the possession of a receptaculum seminis, and in the extent and position of the cirrus sac. It agrees in all important particulars with the description of the sub-family *Psilostominae*, Lühe, 1909, but differs in the branched nature of the testes: the genus *Psilochasmus*, Lühe, 1909, however, has somewhat deeply lobed testes. As the present form differs in important characters from any existing genus, it is necessary to create a new one for its reception. For this I propose the name *Testifrondosa*, and for the species *T. cristata*.

Testifrondosa (n.g.)

DIAGNOSIS :—

Psilostominae : Body covered with scales. Oral sucker smaller than the ventral, latter drawn out posteriorly into a sac-like prolongation. Prepharynx small, pharynx small, globular. Oesophagus short. Intestinal caeca nearly reaching posterior end of the body. Genital pore near intestinal fork. Cirrus sac much elongated, extending beyond ventral sucker and containing vesicula seminalis. Pars prostatica and Laurer's canal present. Testes branched, in posterior half of the body, one behind the other. Shell gland central. Ovary anterior to testes. Receptaculum seminis present. Vitellaria lateral, meeting in the middle line posterior to testes. Uterine coils between shell of gland and cirrus sac. Excretory canal pear-shaped. Eggs large, operculated.

HOST : *Sus cristatus* (Wagner, 1909).

TYPE SPECIES : *Testifrondosa cristata*, n.sp.

***EURYTREMA DAJII*, n. sp. (Plate VIII)**

From thirty to forty specimens of this species were collected in the bile-ducts of a *Bos indicus* also infested with *Paramphistomum cervi* and *Fasciola gigantica*. They were 5 to 6·7 mm. long by 3·5 to 4 mm. broad, and 6·32 mm. thick, coloured red and very sluggish. All were mature. They appeared to be roundish in their natural habitat, but when put in luke-warm water became leaf-like and flattened dorso-ventrally. The body was transparent, so that upon applying a little pressure in the living condition, the whole anatomy could be made out under a low magnification. The body is covered with a thin cuticle, bearing small square scales 20μ to 50μ, very thinly distributed and absent from the edges. The maximum

breadth is reached at the level of the ovary or a little behind it, thence the body narrows towards the anterior extremity, ending, however, rather bluntly owing to the presence of the oral sucker. Above this the margin of the body projects in the shape of a thick lip. At the posterior end is a conspicuous tongue-like appendage—the caudal appendage. A little behind the anterior extremity is a circular mouth surrounded by an oral sucker which, in most cases, is round, but sometimes has its antero-posterior diameter slightly the bigger. On an average the oral sucker is 750μ in diameter. The ventral sucker lies in the anterior half of the body: its size in some cases is equal to, and in others slightly larger than that of the oral sucker, the greater diameter being on the average 850μ. The ratio between the two suckers is 1 : 1·3; the distance between their centres is a little more than one-third the total length of the whole body. The pharynx is small, 250μ by 220μ and lies immediately behind the oral sucker. Following it is a very short oesophagus approximately 50μ long. This immediately bifurcates into two intestinal caeca which diverge and pass along the sides of the body in a sinuous course that becomes specially accentuated behind the testis. Their blind ends terminate a little in front of the caudal appendage, about 800μ from the posterior end of the worm. The excretory pore is at the extreme posterior end, on the top of the caudal appendage. It leads in to an excretory vesicle which becomes bigger as it passes anteriorly and divides into two branches at about half-way between the posterior border of the ventral sucker and the hinder end of the body. These branches pass slightly anteriorly to each side and cross over the intestinal caeca. At the level of the ovary each divides again into anterior and posterior branches which can be traced almost to the ends of the body.

The genital pore is a little behind the intestinal fork, nearer the oral than the ventral sucker. The cirrus sac is elongated, much wider anteriorly and tapering behind, and extends from a little behind the intestinal fork to a little in front of the ventral sucker. It is inclined slightly to the left of the middle line. In a fully extended specimen it is 1 mm. long by 0·38 mm. broad, but the size varies greatly according to the degree of body contraction. The testes almost touch the intestinal caeca behind the ventral sucker. They measure 400μ to 560μ in length and 220μ to 350μ in breadth.

Their position varies greatly ; sometimes they are quite symmetrical, but not infrequently the right is a little in advance of the left or *vice versa*. The margin is lobed, sometimes deeply, sometimes slightly, the number of lobes being usually four. From the anterior lobe or from the lobe directed towards the ventral sucker of each testis, arises a vas efferens which runs inwardly towards its fellow on the other side and joins with it to form a vas deferens at the base of the cirrus sac. The vas deferens then passes into the cirrus sac where, after a short interval, it widens into a vesicula seminalis. The vesicula seminalis continues as a convoluted tube filling up nearly two-fifths of the posterior part of the cirrus sac, then, as a ductus ejaculatorius and finally as a muscular cirrus, opens into the genital sinus by the male aperture. The ductus ejaculatorius is surrounded by the pars prostatica.

The ovary is small, 220μ to 300μ by 170μ to 250μ. It lies in the posterior half of the body, a little to the left of the middle line and a short distance behind the testes. In a well extended form it appears to be trilobed, in others its shape varies, sometimes being oval, and sometimes elongated transversely. From its inner side a short duct passes into the shell gland, 200μ by 130μ, near the ovary. On the dorsal side and overlapping the ovary is a small round receptaculum seminis, 190μ by 160μ. Laurer's canal proceeds from the ovary anteriorly towards the ventral sucker, a little behind which it terminates. In a fully extended condition it is 300μ long by 30μ wide. Its most striking characteristic is the absence of an external pore. In living specimens its blind nature can be ascertained by the movements that it makes when subjected to slight change of pressure : even in sections no opening to the exterior could be discovered. In this respect the present species differs from all others of the same genus.

The uterus becomes visible after the shell gland. It first coils posteriorly, then passes anteriorly between the right testis and the ventral sucker to continue, still coiled, on the right side of the latter, where it joins a long, thin, muscular vagina. This passes lateral to the cirrus sac and opens by the female aperture on the anterior side of the genital atrium. The uterine coils are numerous and nearly overlap the intestinal caeca : anteriorly they are limited by the testes and the ventral sucker : posteriorly they may end at the

termination of the intestinal caeca, but more often project beyond them and occupy a portion of the caudal appendage. The uterine coils are not in well-defined loops but appear to be scattered branches. Sometimes, in a particular state of contraction, the descending and ascending parts of the uterus pass so near one another in the middle line, that the appearance is that of a central stem with many lateral branches.

The vitelline glands are not well developed. They consist of from ten to thirteen groups on each side, each composed of numerous slender acini. In fully extended specimens the groups lie one behind the other outside the intestinal caeca : they are sometimes round, but are liable to much variation in contracted specimens ; in these the linear arrangement of the groups is disturbed, so that they lie irregularly outside the intestinal caeca, occasionally with the follicles pushed below each caecum. The individual groups of follicles become sometimes so much stretched that the acini are arranged in irregular lines, usually commencing on the outer border of the testis and ending at about half-way between the posterior border of the ventral sucker and the base of the caudal appendage. Sometimes the glands on the same side as the ovary extend further anteriorly than those of the opposite side, and sometimes *vice versa*. Two smaller vitelline ducts, anterior and posterior, from each gland unite to form a transverse vitelline duct which, arising from the centre, passes internally to meet with its fellow from the other side and open into the shell gland. No yolk reservoir was noticed. The eggs are small, operculated, 32μ to 40μ long by 22μ to 27μ broad.

Up to the present, seven species of the genus *Eurytrema* have been described, one of them, *Eurytrema crucifer* (Nicoll, 1914), later being placed by Kossack (1910) in his new genus *Paradistomum*. The description of *Eurytrema parvum* (Senoo, 1907) is not accessible to me in India. From the table given below it may be seen that the present species is allied to *Eurytrema pancreaticum* (Janson, 1889) with which it agreees :

(i) in the possession of a caudal appendage ;
(ii) the position of the genital aperture ;
(iii) the extent and the number of groups of vitellaria, and
(iv) the nature and disposition of the uterus,

but differs

(i) in the possession of cuticular scales ;
(ii) the ventral sucker being slightly larger than the oral ;
(iii) the position of the cirrus sac, and
(iv) the blind nature of Laurer's canal.

These points seem to me of sufficient importance to justify the creation of a new species for which I propose the name *Eurytrema dajii*.

SPECIFIC DIAGNOSIS.

Eurytrema : 5 to 6·7 mm. by 3·5 to 4 mm. Cuticle covered with scales. Caudal appendage well developed. Ventral sucker slightly larger than oral. Genital pore posterior to intestinal bifurcation. Cirrus sac not reaching anterior margin of ventral sucker. Vitellaria elongated, 10 to 13 groups, extending from testis to half the distance between ventral sucker and proximal end of caudal appendage. Laurer's canal with no external opening. Uterus passing anteriorly, lateral to ventral sucker : uterine coils asymmetrical, not crossing intestinal caeca.

HOST : *Bos indicus* (bile ducts).

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TABLE I.

A comparative Table of the species of the genus *Eurytrema*

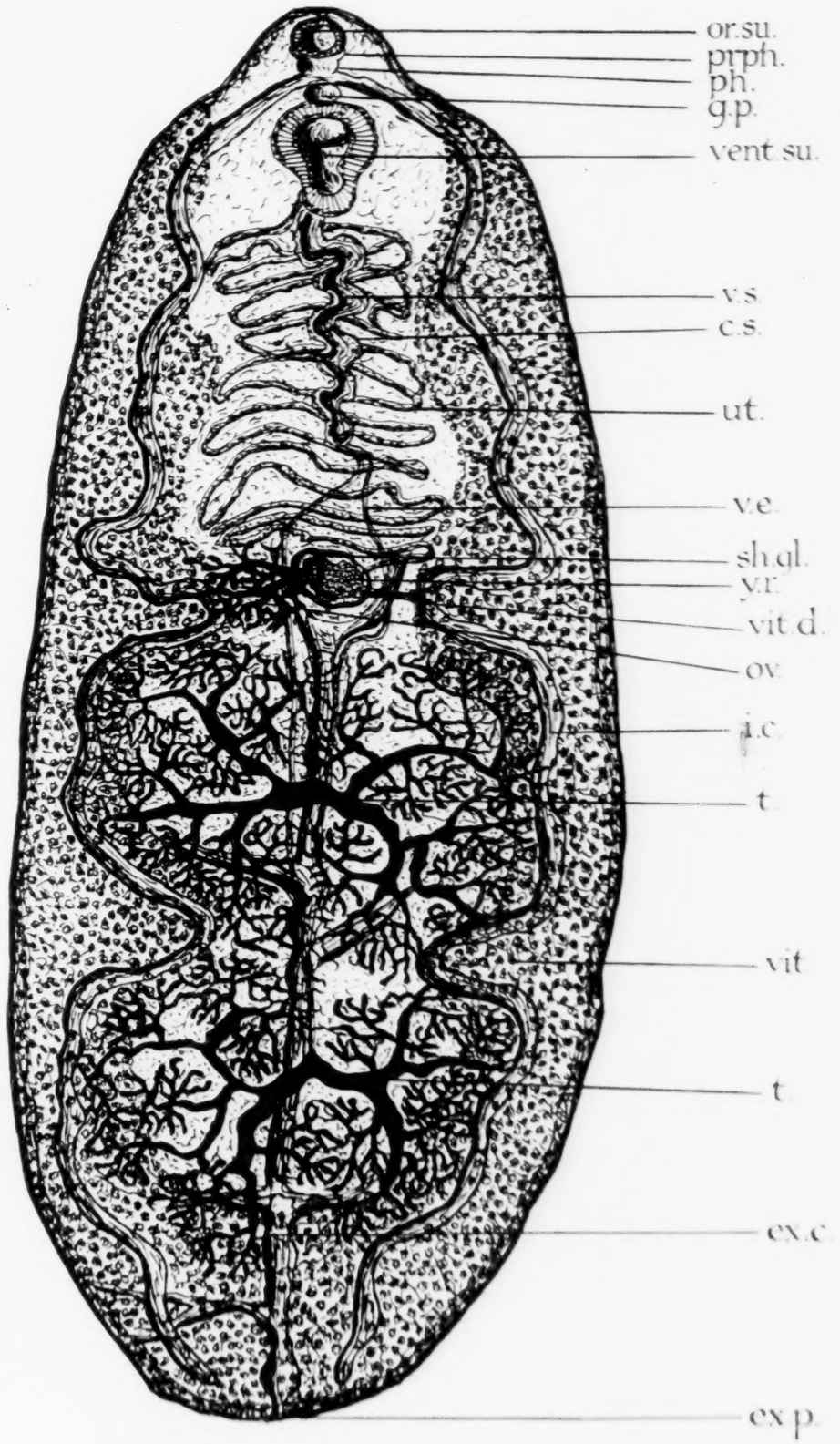
Species	*E. dajii*, n.sp.	*E. pancreaticum* (Janson, 1889)	*E. coelomaticum* (Giard and Billet, 1892)	*E. concinnum* (Braun, 1901)	*E. brumpti* (Railliet, Henry, and Joyeux, 1912)	*E. satoi* (Kobayashi, 1915)
Host	*Bos indicus*, bile ducts	Bovidae, pancreatic ducts	Bovidae, pancreatic ducts	*Vivera zibetha*, gall bladder	Chimpanzee, bile and pancreatic ducts	*Macacus cynomolgus*, pancreatic ducts
Size	5-6·7 by 3·5-4 mm.	13-14 by 6·5-7 mm.	7-10 by 4-5·5 mm.	2·7-3 by 1·6 mm.	3·5-4 by 1·8-2·3 mm.	6-6·5 by 2-3 mm.
Scales	Present	Absent	Absent	Absent	Very fine	Absent
Caudal appendages	Present	Present	Present	Absent	Absent	Less conspicuous
Size of suckers	Ventral slightly larger	Oral larger	Both equal	Ventral larger	Ventral larger	Ventral slightly larger
Genital pore	Behind bifurcation of intestine	Behind bifurcation of intestine	Behind bifurcation of intestine	Before bifurcation of intestine	Before bifurcation of intestine	Behind bifurcation of intestine
Cirrus sac	Not reaching anterior border of ventral sucker	Reaching anterior border of ventral sucker	Reaching anterior border of ventral sucker	Not reaching anterior border of ventral sucker	Not reaching anterior border of ventral sucker	Not reaching anterior border of ventral sucker
Vitellaria	Elongated, 10 to 13 groups, from testes to one half the distance between ventral sucker and caudal appendage	Elongated, 10 to 12 groups, from testes to one half the distance between ventral sucker and caudal appendage	Elongated, 6 to 8 groups posterior to testes	Round, 6 groups	Round, 11 to 14 groups behind ovary	Elongated, 3 to 4 groups behind ovary
Laurer's Canal	With no external pore	With external pore	With external pore	With external pore	With external pore	With external pore
Position of uterus	By the side of ventral sucker	By the side of ventral sucker	By the side of ventral sucker	Above ventral sucker	Above ventral sucker	By the side of ventral sucker
Uterine groups	Anterior uterine groups asymmetrical; do not cross over intestinal caeca	Anterior uterine groups asymmetrical; do not cross intestinal caeca	Anterior uterine groups asymmetrical; do not cross intestinal caeca.	Anterior uterine groups slightly asymmetrical; do not cross intestinal caeca	Anterior uterine groups asymmetrical; cross intestinal caeca	Cross intestinal caeca

Eurytrema parvium (Senoo, 1907) is not included in the above table.

EXPLANATION OF PLATE VI

Ventral view of *Fasciolopsis fülleborнii*

c.s.	Cirrus sac.	*sh.gl.*	Shell gland.
ex.c.	Excretory canal.	*t.*	Testis.
ex.p.	Excretory pore	*ut.*	Uterus.
g.p.	Genital pore.	*v.s.*	Vesicula seminalis.
i.c.	Intestinal caecum.	*v.e.*	Vas efferens
or.su.	Oral sucker.	*vent.su.*	Ventral sucker.
Ov.	Ovary.	*vit.*	Vitellaria.
ph.	Pharynx.	*vit.d.*	Vitelline duct.
prph.	Prepharynx.	*y.r.*	Yolk reservoir.



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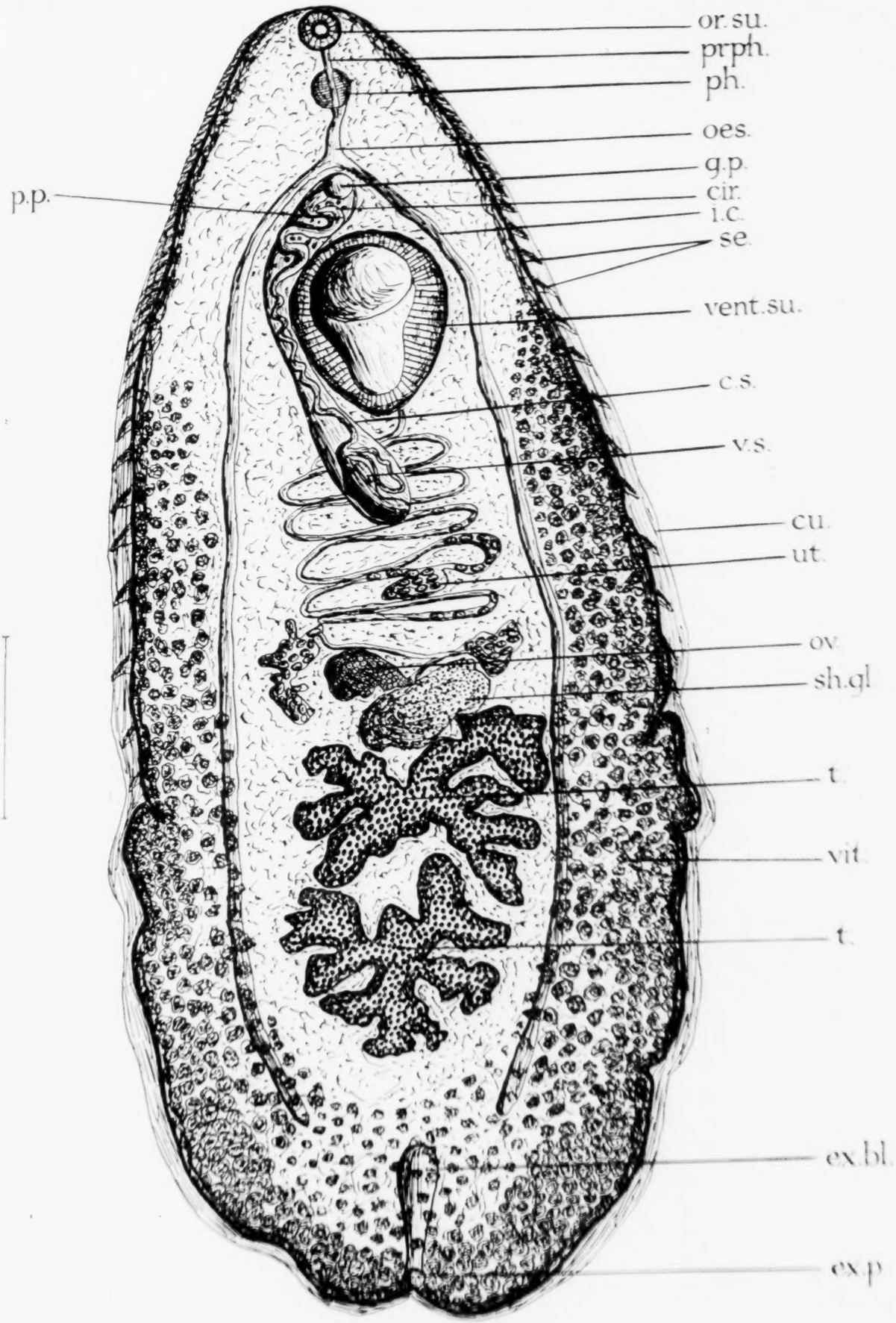
EXPLANATION OF PLATE VII

Dorsal view of *Testifrondosa cristata*.

c.s.	Cirrus sac.	*p.p.*	Pars prostatica.
cir.	Cirrus.	*prph.*	Prepharynx.
cu.	Cuticle.	*ph.*	Pharynx.
ex.bl.	Excretory bladder.	*sc.*	Scales.
ex.p.	Excretory pore.	*sh.gl.*	Shell gland.
g.p.	Genital pore.	*t.*	Testis.
i.c.	Intestinal caecum.	*ut.*	Uterus.
Oes.	Oesophagus.	*v.s.*	Vesicula seminalis.
or.su.	Oral sucker.	*vent. su.*	Ventral sucker.
ov.	Ovary.	*vit.*	Vitellaria.



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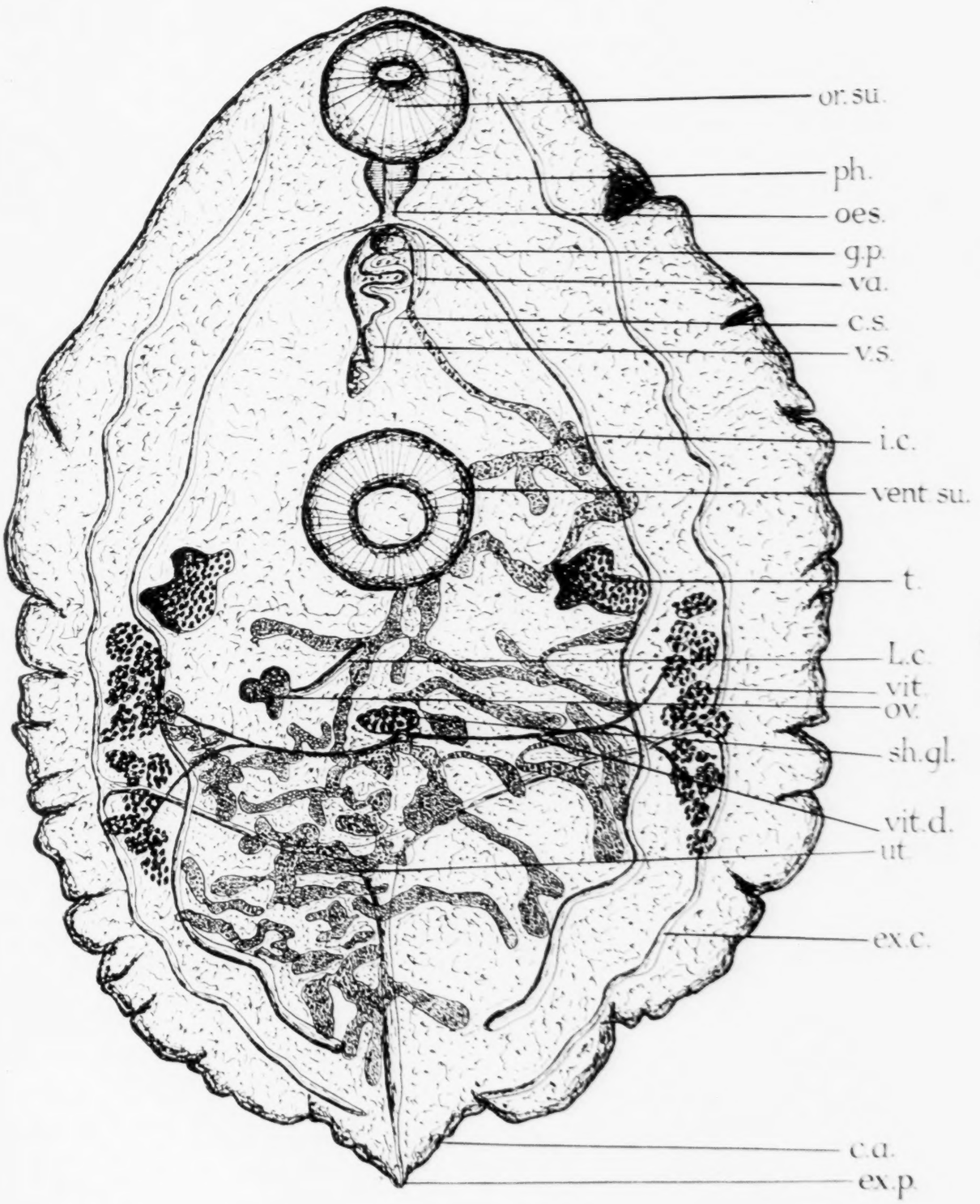
EXPLANATION OF PLATE VIII

Dorsal view of *Eurytrema dajii*.

c.s.	Cirrus sac.	*ov.*	Ovary.
c.a.	Caudal appendage.	*ph.*	Pharynx.
ex.c.	Excretory canal.	*sh.gl.*	Shell gland.
ex.p.	Excretory pore.	*ut.*	Uterus.
g.p.	Genital pore.	*v.s.*	Vesicula seminalis.
i.c.	Intestinal caecum.	*va.*	Vagina.
L.c.	Laurer's canal.	*vent.su.*	Ventral sucker.
oes.	Oesophagus.	*vit.*	Vitellaria.
or.su.	Oral sucker.	*vit.d.*	Vitelline duct.



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OBSERVATIONS ON THE CAUSAL ORGANISM OF RAT-BITE FEVER IN MAN

BY

ANDREW ROBERTSON, M.B., Ch.B.

(GROCERS' RESEARCH SCHOLAR AND ASSISTANT IN THE DEPARTMENT OF PROTOZOOLOGY, LONDON SCHOOL OF TROPICAL MEDICINE.)

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In June, 1923, a patient was admitted to the Hospital for Tropical Diseases giving a typical history of rat-bite fever, and showing all the clinical signs of the disease. The clinical aspects of the case have already been published in detail by Dr. G. C. Low (1924), Senior Physician, Hospital for Tropical Diseases, and the following observations have been made on a strain of the parasite derived—thanks to the courtesy of Dr. Low—from this patient.

On 11th June, 1923, two rats and two mice were inoculated with citrated blood from the patient, who was then at the height of a pyrexial attack, the former being given about 3 c.c. each, and the latter 1·5 c.c. all intraperitoneally. Of the four animals inoculated, only one mouse showed the parasite in its peripheral blood. These animals had been bred in the laboratory, and examinations of their blood for several days previous to the inoculation failed to reveal parasites. The appearances and behaviour of the parasite in this mouse were such as to indicate that the organism differed in many respects from the members of the genera *Spirochaeta*, *Leptospira*, etc. to which it has at times been referred, and accordingly search was made through the literature for previous descriptions.

HISTORICAL

Vandyke Carter (1887) published a description of a *Spirillum*, which he discovered in the blood of a rat (*Mus decumanus*), in India. His attention was first drawn to the possible presence of some parasite by a quick, twirling movement of the red blood corpuscles in

fresh blood, and, on further investigation, he found that this was due to an organism, which he described as an

'extended and uniformly slender filament of clearly spiral construction, having a length commonly somewhat less than the diameter of a blood disc, but varying from 5μ to 9μ . . . . and according to its length presenting from four to eight close spiral turns.'

He was unable to make out the presence of flagella at the ends. The movement of the organism was very active, and consisted of

'rotation round the long axis, propulsion either forward or backward, and occasionally an energetic twisting or lashing.'

From its morphology, movements, and behaviour to reagents Carter concluded that the organism in question was

'a bacterium belonging to the genus spirochaete . . . . such as from its small dimensions might be named provisionally *Spirillum minor*.'

Attempts to inoculate the organism into another rat and also into a monkey were not successful.

There seems to be little doubt that the organism, which Carter found in the blood of the rat, is the same as that which causes rat-bite fever in man.

Thereafter there occur numerous mentions of similar organisms, which were found in the blood of animals. Lingard (1899) found a small *Spirillum* in the blood of a bandicoot (*Mus giganteus*). This he was able to inoculate into rabbits and guinea-pigs, but in the latter it was possible to demonstrate the organism only in the final stages of the infection. While Lingard's description of the parasite is lacking in detail, its behaviour in inoculated animals is similar to that of the virus of rat-bite fever as reported by various workers.

Borrel (1905) found a spirochaete in cancer growths of mice. This work was confirmed by Calkins and Clowes (1905), Deetjen (1906) Tyzzer (1906-07), and Gaylord (1907). There is a marked similarity between the organisms described by these workers, although the measurements varied considerably.

Wenyon (1906) described a spirochaete in the blood of mice which he named *Spirochaeta muris*. This he considered to be identical with the spirochaete found by Borrel, but with reference to the organism of Carter he states that

'since the morphological characters of spirochaetes are not sufficient to establish the identity of any form it is necessary to rely on other characters, notably their behaviour

in various hosts, and their pathogenic or other action. As nothing is known of Carter's *Spirillum* of the rat apart from its morphology, the spirochaete of the mouse must be considered to be new to science.'

Morphologically, Wenyon's *Spirochaeta muris* closely resembled Carter's *Spirillum*, and the characteristic movements were also present. Wenyon was unable to demonstrate flagella, but suspected that they might be present, since all the movements of the organism seemed to indicate their presence. The spirochaete was easily inoculable into mice, but could not be demonstrated in guinea-pigs.

Breinl and Kinghorn (1906) isolated a spirochaete from the same source as Wenyon, viz.:—a mouse inoculated with *Trypanosoma dimorphon*, which was sent to them from Paris. They also found a similar spirochaete in the blood of a wild mouse. The parasite was smaller in size than that described by Carter, and accordingly, since they considered it to be a distinct organism, they named it *Spirochaeta laverani*.

MacNeal (1907) demonstrated the presence of flagella at the ends of the spiral in a strain of the parasite isolated from one out of thirty-nine rats (*Mus decumanus*), caught at Morgantown. He considered this organism to be identical with those of Carter, Wenyon, Breinl and Kinghorn, and Nicolle and Compte (in a bat). Despite the fact that he thought his organism to be the same as that of Carter, and that terminal flagella had been seen, MacNeal adopted Wenyon's name *Spirochaeta muris* (var. *Virginiana*).

In man, rat-bite fever has long been recognised as a definite clinical entity. Early accounts of the disease were given by Wilcox (1840) in America, Millot-Carpentier (1884) in France, Peña y Maya (1885) in Spain, Miyake (1889) in Japan, and Horder (1909) in England. While it was soon recognised that the disease depended on the introduction of some virus into the wound caused by the bite of the rat, nevertheless it was comparatively recently that it was associated with a definite organism. That more than one disease may be inoculated into man by the bite of a rat is practically certain, and this led to some confusion, in that some of these diseases were mistaken for the fever of the relapsing type, which is the clinical entity known as rat-bite fever. Shikami (1907) attributed rat-bite fever to a member of the Telosporidia; Middleton (1910) thought that the causative organism was a *Diplococcus*;

Ogata (1911), who produced rat-bite fever experimentally by allowing a rat to bite guinea-pigs, considered that the disease was due to an *Aspergillus;* Proescher (1911) described *Bacilli,* which were very numerous in the base of the wound; Schottmüller (1914), Blake (1916) and Tileston (1916) isolated from the blood of patients, who had been bitten by rats and by a South African squirrel, a *Streptothrix,* which was named *Streptothrix muris ratti.* With reference to this last, it is of interest that Tunnicliff (1916) isolated a similar *Streptothrix* from rats with broncho-pneumonia. Hata (1912), Surveyor (1913) and Dalal (1914) reported cures by the use of salvarsan, arguing that, on account of the periodicity of the fever and the peculiar eruption, the infecting organism might be allied to the *Spirochaetae.*

It was, however, left to Futaki (1915) and his co-workers to discover the causative organism of rat-bite fever in man. This was a spiral organism having flagella at the ends, varying in length from 2μ to 5μ, and when the flagella are included from 6μ to 10μ. They named it *Spirochaeta morsus muris,* and considered it to be of the nature of a *Treponema.* The movements of *S. morsus muris* were very rapid, ' resembling those of a vibrio.' It stained readily with Giemsa's stain. Animal experiments were successful; monkeys, mice, house rats and white rats were inoculated with positive results. These authors also claim to have cultivated the parasite on Shimamine's medium, but the culture organisms, as described and figured, differed very considerably from the forms found in man and animals. In cultures, forms appeared up to 19μ in length, and having at times as many as nineteen coils. The coils or spirals were wider than those of the blood forms—one coil to 2μ, whereas the blood forms had one coil to 1μ. Whether it was possible successfully to inoculate these cultural forms into experimental animals is not stated. Further mention of these cultural forms will be made later.

Futaki and his associates were of the opinion that the organism, which they had isolated, differed in certain respects from those previously described as occurring in the blood of mice, rats and other animals, and further, that, as no connection between these organisms and rat-bite fever in man had been demonstrated, they were justified in considering that the parasite was a new species.

Ishiwara, Ohtawara and Tamura (1915) dealt with certain

experimental aspects of the question. They allowed infected rats to bite guinea-pigs, thus confirming the work of Ogata, and studied the course of the disease in these animals. They discussed the similarity of Wenyon's *Spirochaeta muris* and the *Spirochaeta laverani* of Breinl and Kinghorn to *S. morsus muris*. Ishiwara and his associates did not succeed in cultivating it.

Kaneko and Okuda (1917), dealing with the organism in man, came to the conclusion that the long and short forms described by Futaki were not diverse in type. The short forms were found in the peripheral blood, but the long forms—similar to the cultural forms of Futaki—they found in renal casts, in the tissues of the kidneys and in the suprarenals. This change in the morphology they attribute to the formation of immune bodies in the patient, and are of the opinion that the long forms are really old and degenerate individuals.

Ido, Ito, Wani and Okuda (1917) obtained blood serum from three human patients recovering from rat-bite fever. The effect of this serum they tested on spirochaetes in the blood of guinea-pigs, which had been infected from a wild rat, *Mus decumanus*, and found that the organisms were killed, whereas in controls treated with serum from patients who had not had rat-bite fever, and also with isotonic salt solution, the spirochaetes remained actively motile and relatively numerous. This is of interest in that, in addition to illustrating the action of immune serum on the parasites, it suggests a certain degree of association between the causative organism of rat-bite fever in man, and similar parasites found in the peripheral blood of mice and rats.

Row (1917) isolated a spiral organism from a case of human rat-bite fever in India. This was rather smaller in size than that described by the Japanese workers, 3μ to 5μ in length, and Row thought it a distinct species. Later, in 1922, Row named his organism *Spirochaeta petit*. Row found that

> 'the broad distinction made by Futaki into the long and short forms according to the situation from which the virus is derived—the long forms from lymphatic nodules and short ones from the peripheral blood—does not hold good in Bombay.'

The Bombay virus was not fatally virulent to guinea-pigs and rats. Row came to the conclusion that the number of spirochaetes in the organs of infected animals was not greater than could be accounted

for by the blood supply to these organs, and that such as were found were uniform in type with those present in the peripheral blood. The Bombay virus was shorter than that described by the Japanese workers, and was more slender than English strains. The fact, too, that at first the flagella of the Bombay strain were not seen, was an additional reason why Row should place his spirochaete in a new species. Parmanand (1923), however, demonstrated the terminal flagella in this strain, and came to the conclusion that it was similar to the *Spirochaeta morsus muris* of Futaki.

Izumi and Kato (1917), by serological methods, proved the relationship of the spirochaete found in cases of cat-bite disease to that of rat-bite fever.

Manson-Bahr (1922), in ' Manson's Tropical Diseases,' referred the causative organism of rat-bite fever to the genus *Leptospira*, and retained the specific name *morsus muris*. His reasons for making this alteration in the nomenclature were not stated. Sangiorgi (1922) suggested the generic name *Treponemella* for the rat-bite virus.

Numerous reports of cases of rat-bite fever have appeared in the literature since the discovery of the parasite by Futaki and his associates, and the foregoing is not intended to be an exhaustive survey of the literature on the subject. As many of those communications, which have a direct bearing on the parasite, its morphology, nomenclature, etc., as possible, have been mentioned, but many other papers on the subject will be found in the list of references.

THE PRESENT CASE

While the patient was having one of his periodical bouts of fever, specimens of his blood, saliva, and urine were examined. In the blood, both in stained preparations and in fresh preparations examined with dark ground illumination, no organisms were found. In the urine, also, the results of the search were negative. The saliva proved to have the usual number of spirochaetes of all kinds, but there was also present an undoubted *Spirillum*, which, however, was so scarce that accurate observations could not be made. At the time of the examination the patient showed the typical, dark purplish-red, exanthematous rash, especially over the lower ribs

and upper part of the abdomen on the right side. One of the most conspicuous patches of the rash was chosen, and its surface scarified. Gentle pressure was applied to the scarified area, and preparations of the exuded serum were examined with the dark ground. In one specimen only were the organisms found. Stained films from this source were negative.

A strain of the parasite was isolated in the blood of mice, which had been inoculated with citrated blood from the patient.

MORPHOLOGY

The body of the organism, which is coiled into a more or less perfect spiral, is rigid. Occasionally, under certain circumstances, the body may be bent, but as soon as the factors which cause the bending are removed, it returns to the original state. In comparison with the members of the genus *Spironema* this rigidity is most striking. The *Spironemata*, in addition to the general flexibility of their bodies, progress by means of an undulating movement, which passes from one end of the body to the other ; the rat-bite organism, on the other hand, retains its fixed shape during movement, although in dark ground preparations the rapid spinning of the body may simulate a wave-like mode of progression. At each end of the spiral body are flagella, usually single but sometimes multiple, and their function seems to be not so much to propel the organism, as to produce the spinning movement of the body, which then passes through the fluid much in the same manner as a screw enters wood. Movement, which is very rapid, is usually in more or less of a straight line, but sometimes consists of sudden dashes to and fro, or in any direction, when either end of the body may be, for the time being, the anterior. Lashing movements of the body are also seen, but there is a possibility that this may be due to the artificial conditions under which the observations are made, such as the fixation of the flagellum at one end to the under surface of the cover glass, to a group of red blood corpuscles, etc.

With regard to the size of the parasite considerable variations have been noted. Thus it varies in different animals, and also in the same animal from day to day. There appears to be a certain tendency to uniformity of size in any given animal on any given day, by which

is meant that one day the preponderating forms will be, for example, long forms, whereas the following day, or subsequently, the majority observed will be shorter or medium-sized. The largest forms seen were between 14μ and 15μ including the flagella, *i.e.*, slightly more than double the diameter of the red blood corpuscles of the mouse, and, when body length only is considered, the maximum measurements were between 9μ and 10μ. It should be noted that the length of the flagella is by no means proportionate to the dimensions of the body, as small forms with a body-length of only 2·5μ may have flagella as long as individuals with a body-length of 8μ or thereby. The smallest individuals measured 3·25μ, but these had a flagellum at one end only. The smallest body-length was 1·5μ. The average size was between 3μ and 5·5μ, and including the flagella between 6μ and 8μ.

The number of coils or turns of the body varies from one-and-a-half to eight or nine. The width of the body, owing to its spiral construction, is extremely difficult to ascertain with accuracy. Fixed and stained preparations are of little value in this respect, as the degree of distortion is very great. The average width is about 0·2μ, and the width of the spiral as a whole about 0·7μ.

Division takes place by transverse binary fission of the body into two, more or less equal portions. A constriction appears about the middle of the body, and this gradually deepens until the two parts are connected by the merest thread. Certain peculiarities of movement may be seen in these dividing forms owing to the different alignment of the two halves. Finally, the two portions separate, giving rise to two new individuals, which, at first, have flagella at one end only. Later a flagellum appears at the other end. It seems probable that the fully developed organism has a flagellum at each end, and that those forms with flagella at one end only are the products of division. In stained preparations it is quite common to find the flagella at each end to be multiple, but this seems to be more the result of the fixation than a characteristic of the parasite, as, in fresh specimens in which the movement has slowed, it has only rarely been possible to demonstrate more than one flagellum. For some reason, the flagella are very difficult to stain, and even using the same technique, constant results cannot be obtained. Several stains have been tried and various methods of fixation, but the best results

have been obtained with slight modifications of Leishman's and Giemsa's stains.

Futaki and his co-workers described two forms of the parasite, the first as found in the peripheral blood, the second longer forms in cultures. Kaneko and Okuda mentioned similar long forms, which they found in the kidneys, suprarenals and renal tube casts, and these they interpreted as older and somewhat degenerate individuals. Morphologically, there is a great difference between the cultural forms and the blood forms, the former being as many as 19μ in length and having coils twice as wide as the blood forms, and indeed this variation, especially in view of the fact that no one has succeeded in cultivating the rat-bite virus since Futaki, is so marked that it is necessary to bear in mind the possibility that in the infected animals and cultures, there were spirochaetes other than that of rat-bite fever. In the present case careful search was made in the tissues of animals which were killed during their convalescence from the disease, and also at various stages in the course of the infection, with the result that no true spiral organisms were found other than the forms described above as occurring in the peripheral blood. Hogue (1924), working on *S. eurygyrata*, has shown that the cultural forms of a spirochaete may differ markedly in their morphology from the forms found under natural conditions, but even this is insufficient to explain the difference in length, shape, structure, etc., between the blood forms and Futaki's cultural forms. It is significant, also, that it was not possible to inoculate successfully experimental animals with the cultures.

CULTIVATION EXPERIMENTS

In the present case all attempts to cultivate the virus have so far proved unsuccessful. N.N.N. (Novy, MacNeal, Nicolle), Noguchi's and Shimamine's media and a dilute blood agar were the chief media used. These were incubated at different temperatures but without result. The cultural experiments are being continued.

ANIMAL EXPERIMENTS

Of four animals inoculated from the patient, only one mouse showed the parasite in its peripheral blood on the seventh day after inoculation. The other mouse and the two rats never showed

parasites. Mice proved to be most convenient animals in which to carry on the infection, because the *Spirilla* appeared in such relatively large numbers in their blood, and also because they seemed to be little incommoded by the disease. The average length of time which elapsed after inoculation and before parasites appeared was five days, and the infection reached its maximum intensity, so far at least as numbers were concerned, on the ninth day after inoculation. It was quite common, however, for the parasites to show up in fair numbers about the fifth day, disappear the following day, and return in increased numbers on the seventh or eighth day, when they remained constantly present for some weeks. No explanation of this temporary disappearance is known. The shortest period before *Spirilla* could be detected was three days, and the longest fourteen days. Having so quickly reached its maximum intensity the infection gradually declined, the numbers in the blood becoming less and less, until finally they disappeared entirely. As a rule, it is possible to recover the strain by inoculating the blood of the convalescent mouse, even after the *Spirilla* cannot be demonstrated microscopically owing to their scarcity, into a fresh animal. The infection usually persists for the matter of six weeks or so, but, in this instance at least, considerable trouble has been experienced by the mice dying from intercurrent affections.

Guinea-pigs, when inoculated with this strain, showed symptoms of disease. A few days after inoculation, usually about two-and-a-half to three days, there was a definite rise in temperature, which was followed at irregular intervals by similar febrile attacks. During this period, which usually lasted between twenty-one and twenty-four days, the animals were definitely ill, as shown by loss of appetite, poor condition of the fur, general weakness, etc. At the end of this time the disease seemed to reach its most crucial point, and the guinea-pigs were very ill indeed, but this was followed by a gradual convalescence, and in no instance did the animals die from the infection.

It was not possible to demonstrate the *Spirilla* in the blood of the guinea-pigs at any stage, nor were mice successfully inoculated with their blood. These findings are of interest in comparison with those of other workers. Certain strains have proved to be lethal to guinea-pigs, whereas others have caused them little inconvenience ;

some strains have shown parasites in the peripheral blood over a comparatively long period, while others have only appeared immediately before the death of the animal or not at all. One of the guinea-pigs was inoculated subcutaneously in the leg, and this was followed by a painless, oedematous swelling, which persisted until the twenty-first day after. This animal also showed symptoms of the generalised disease.

After the guinea-pigs had recovered, it was found that their serum, when mixed in equal quantities with the blood of mice containing *Spirilla*, rendered the parasites immobile in twenty minutes, whereas in control experiments, the blood of the mice being mixed with serum from uninfected guinea-pigs, with serum from guinea-pigs with various strains of trypanosomes, and with normal saline, the organisms were as actively motile after two hours as they appeared to be when the blood was freshly drawn. The immune bodies in the serum do not seem to be very powerful, since, in dilutions less than about one in four, their effect on the *Spirilla* was very slight. So far no signs of agglutination of the parasites have been seen, although this phenomenon has been described. Mouse blood containing *Spirilla* was mixed with equal parts of immune guinea-pig serum and allowed to stand for fifteen minutes, and was then inoculated into a fresh mouse. Mice, which were inoculated at the same time with untreated blood, showed a heavy infection in five days, whereas this one did not show infection till a considerable time after, and even then the parasites were so few in number that it was only by careful search that they could be demonstrated.

Of the two rats which were inoculated with the blood from the human patient, one died in a few days, while the other never showed parasites in its blood or any signs of disease. It has been possible to find parasites in scanty numbers in the blood of young inoculated rats, but, unless the rats were very young indeed, the *Spirilla* never became evident.

DISCUSSION

It has been suggested by several workers that the rat-bite fever organism is more closely allied to the genus *Spirillum* than to the *Spirochaeta*, and that is the opinion arrived at by the study of the present strain.

Dobell (1918) discussed the nomenclature of the spirochaete of syphilis. He pointed out that this parasite was morphologically so different from the type species of the genus *Spirochaeta*, viz. :— *S. plicatilis*, that it could not be placed in the same genus. The rat-bite fever organism, with its non-flexible body, terminal flagella and absence of an axial filament, is even more divergent in character, and accordingly cannot be referred to the genus *Spirochaeta*.

For practically the same reasons the rat-bite organism cannot be placed in the genus *Spironema*, the type species of which is *S. pallidum* (Schaudinn, 1905) Vuillemin, 1905.

Manson-Bahr (1918) named the rat-bite fever parasite *Leptospira morsus muris*. He did not state his reasons for thus altering the generic name, but it seems probable that he was influenced, firstly, by the cultural forms of Futaki, which, as figured, have some resemblance to *Leptospira*, and, secondly, by the forms described by Kaneko and Okuda in tissues, renal casts, etc. Now it has been shown by Hogue (1922), working on *S. eurygyrata*, that the cultural forms of a spirochaete may differ from the forms found in nature to a considerable degree, but the dissimilarity of the blood forms and Futaki's cultural forms is too marked to be explained on this basis. With regard to the forms found in tissues, careful search has been made in the organs of animals infected with the present strain, which have been killed at various stages of the infection and also during convalescence, without finding any spiral organism other than the form as described in the peripheral blood. This is in agreement with the findings of Row and other workers. In the present case, in attempting to cultivate the parasite on Shimamine's medium, numerous filaments were found, which closely simulated the appearances of *Leptospira* and of *Spironemata*. It therefore seems probable that Kaneko and Okuda have been dealing with some spirochaetal infection superimposed on that of the rat-bite fever organism, and that Futaki and his co-workers have either cultivated some other organism, or have mistaken the filamentous threads in the medium for true spirochaetes. The work of Thomson (1923) on pseudo-spirochaetes may have some bearing on this question.

The conclusion must be arrived at that the rat-bite fever organism with its terminal flagella, and non-flexible, non-undulating, spiral body should be referred at present to the genus *Spirillum*, Ehrenberg, 1830.

In the consideration of the specific name, which should be applied to the rat-bite fever organism, the first point to be decided is whether more than one distinct species of *Spirillum* has been described in the blood of mice and rats, bandicoots, etc., and if not, whether this is the same as that causing rat-bite fever. Firstly, as regards the *Spirilla* in the blood of mice, rats, etc., the prior discovery of such an organism rests with Carter. That Carter was dealing with a true *Spirillum* seems clear from his description of the morphology and of the characteristic movements. Following Carter, the *Spirochaeta muris* of Wenyon, with which can be grouped the *Spirochaeta laverani* of Breinl and Kinghorn as it came from the same source, was the next to be given a specific name. That this was a *Spirillum* also there is now no doubt, and so far as its morphology is concerned, its variations from *Spirillum minor* are such as may be seen in different strains of the same species. Carter was unable to demonstrate his organism in an experimentally infected rat and monkey, but this is quite in accordance with the findings of later workers using strains of the rat-bite parasite. It is quite impossible to prove that Wenyon, and the large number of workers such as Lingard, Borrel, Deetjen, etc., who found similar spiral organisms in mice and other animals, but did not name them, were not dealing with distinct species, but the contention is that the bulk of evidence, in view of more recent work, is so strongly suggestive as almost to amount to a certainty that they were describing *Spirillum minor*. Admittedly slight differences do occur, in morphology and also in the action on inoculated animals, but similar differences are found in undoubted rat-bite fever strains, and it is suggested that these variations are insufficient to justify the bestowal of new generic and specific names.

The question next arises as to whether the organism isolated by Futaki and his associates is the same as those described in the blood of mice, etc., by the previous workers. In parenthesis it may be said that the *Spirochaeta petit* of Row, in view of the work of Parmanand, is here considered to be the same as the *S. morsus muris* of the Japanese workers, and that, therefore, only one species of *Spirillum* is known to cause true rat-bite fever in man.

The problem resolves itself to this : does there exist in the blood of mice, rats, cats, etc., a *Spirillum* which can be distinguished from other *Spirilla* in the same animals, only by its transmissability to

man ? This, needless to say, is incapable of absolute proof either way, but the submission is that the preponderance of evidence points to the non-existence of a distinct ' human ' species.

1. As regards morphology the forms isolated in human strains cannot be differentiated from the forms in animals naturally infected.

2. Human strains differ from each other in their inoculability into, and reaction in experimental animals. Thus one strain may be highly virulent in guinea-pigs, and show parasites in the animals' blood, whereas another may cause some temporary discomfort only. One strain may be easily inoculable into rats and another may not. Such differences as do occur between human and animal strains in their inoculability and reactions are no greater than may be found in two divergent human strains.

3. It has been proved that serum from patients recovering from rat-bite fever, and also cat-bite disease, contains immune bodies which will immobilise *Spirilla* isolated from naturally infected wild rats.

The conclusion arrived at is that the *Spirillum*, which causes rat-bite fever in man, is the same as the one which occurs naturally in the blood of animals. Following the rule of priority, therefore, the name for the causal organism should be *Spirillum minor*, Carter, 1887. Unfortunately, in naming his organism, Carter did not take into account the fact that the generic name *Spirillum* is neuter. When such a mistake in gender has been made, it is permissible, under the International Rules of Botanical Nomenclature (Art. 57), to make the necessary correction, while the corrected name is cited from the original author and publication. *Spirillum minor*, therefore, becomes *Spirillum minus*, Carter, 1887.

CONCLUSION

1. Up to the present there is no reason for supposing that the *Spirilla* in the blood of naturally infected mice, rats, bandicoots, etc., belong to more than one species.

2. The species of *Spirillum*, which is the causal organism of rat-bite fever in man, is not distinct from the species found naturally in the blood of animals.

3. The correct name for the causal organism of rat-bite fever in man is

Spirillum minus, Carter, 1887.

Synonyms :

Spirochaeta laverani, Breinl and Kinghorn, 1906.

Spirochaeta muris, Wenyon, 1906.

Spirochaeta morsus muris, Futaki, Takaki, Taniguchi and Osumi, 1917.

Leptospira morsus muris (Futaki, Takaki, Taniguchi and Osumi, 1917) emend. Manson-Bahr, 1922.

Spirochaeta petit, Row, 1922.

Treponemella muris (Wenyon, 1906), emend. Sangiorgi, 1922.

Spironema minor (Carter, 1887), emend. Sangiorgi, 1922.

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man ? This, needless to say, is incapable of absolute proof either way, but the submission is that the preponderance of evidence points to the non-existence of a distinct ' human ' species.

1. As regards morphology the forms isolated in human strains cannot be differentiated from the forms in animals naturally infected.

2. Human strains differ from each other in their inoculability into, and reaction in experimental animals. Thus one strain may be highly virulent in guinea-pigs, and show parasites in the animals' blood, whereas another may cause some temporary discomfort only. One strain may be easily inoculable into rats and another may not. Such differences as do occur between human and animal strains in their inoculability and reactions are no greater than may be found in two divergent human strains.

3. It has been proved that serum from patients recovering from rat-bite fever, and also cat-bite disease, contains immune bodies which will immobilise *Spirilla* isolated from naturally infected wild rats.

The conclusion arrived at is that the *Spirillum*, which causes rat-bite fever in man, is the same as the one which occurs naturally in the blood of animals. Following the rule of priority, therefore, the name for the causal organism should be *Spirillum minor*, Carter, 1887. Unfortunately, in naming his organism, Carter did not take into account the fact that the generic name *Spirillum* is neuter. When such a mistake in gender has been made, it is permissible, under the International Rules of Botanical Nomenclature (Art. 57), to make the necessary correction, while the corrected name is cited from the original author and publication. *Spirillum minor*, therefore, becomes *Spirillum minus*, Carter, 1887.

CONCLUSION

1. Up to the present there is no reason for supposing that the *Spirilla* in the blood of naturally infected mice, rats, bandicoots, etc., belong to more than one species.

2. The species of *Spirillum*, which is the causal organism of rat-bite fever in man, is not distinct from the species found naturally in the blood of animals.

3. The correct name for the causal organism of rat-bite fever in man is

Spirillum minus, Carter, 1887.

Synonyms :

Spirochaeta laverani, Breinl and Kinghorn, 1906.

Spirochaeta muris, Wenyon, 1906.

Spirochaeta morsus muris, Futaki, Takaki, Taniguchi and Osumi, 1917.

Leptospira morsus muris (Futaki, Takaki, Taniguchi and Osumi, 1917) emend. Manson-Bahr, 1922.

Spirochaeta petit, Row, 1922.

Treponemella muris (Wenyon, 1906), emend. Sangiorgi, 1922.

Spironema minor (Carter, 1887), emend. Sangiorgi, 1922.

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NOTES ON CERTAIN CESTODES IN THE SCHOOL OF TROPICAL MEDICINE, LIVERPOOL

BY

T. SOUTHWELL

(*Received for publication* 21 *April*, 1924)

Order PSEUDOPHYLLIDEA, Lühe, 1899.
Family *DIBOTHRIOCEPHALIDAE*, Lühe, 1902.
Sub-Family *DIBOTHRIOCEPHALINAE*, Lühe, 1899.

Dibothriocephalus decipiens (Diesing, 1850), Lühe, 1899. One specimen and a fragment from a leopard (*Felis pardus*) Manáos, Amazonas. Collected and presented by Dr. R. M. Gordon. July 10, 1921.

Duthiersia fimbriata (Diesing, 1850), Montic. and Crety, 1891. Several specimens from a monitor Lizard, *Varanus* sp. Madras, India. April 1, 1905.

Order CYCLOPHYLLIDEA, Lühe, 1910.
Family *ANOPLOCEPHALIDAE*, Fuhrmann, 1907.
Sub-family I. *ANOPLOCEPHALINAE*, R. Blanchard, 1891.

Bertiella studeri (R. Bl. 1891) Stiles and Hassall, 1902. One specimen measuring 150 mm. in length, 13 mm. in breadth, and 2 mm. in thickness, from a monkey *Cercopithecus pygerythrus*. Ngoa, North-eastern Rhodesia. July 14, 1912.

Collected and presented by Professor Warrington Yorke, M.D.

The species differs from *B. cercopitheci*, Beddard, 1911, in that the eggs bear a pyriform apparatus, and the pores are regularly alternate.

Bertiella cercopitheci, Beddard, 1911. One immature specimen and a fragment, from *Cercopithecus pygerythrus*, Ngoa, North-eastern Rhodesia, collected and presented by Professor Warrington Yorke, M.D.

Sub-family II. *LINSTOWINAE*, Fuhr., 1907.

Oochoristica truncata (Krabbe, 1879) Zschokke, 1905. Very numerous specimens from the intestine of *Agama colonorum*, Accra, Gold Coast. Collected and presented by Dr. J. W. Scott Macfie, 1912.

Linstowia ameivae, Beddard, 1914. Two fragmented specimens from *Ameivia dorsalis*, Kingston, Jamaica. Collected and presented by Professor R. Newstead, F.R.S.

The specimens agree closely with Beddard's description except in the number of testes.

Beddard states that

'The *testes* lie posteriorly to the vitelline gland, and reach forward on either side of it; they do not, however, extend laterally of the ovary. In a given segment the testes were visible in 18 consecutive sections. The largest number counted in the middle of the series was 43. I therefore calculate the total number to be about 200. The testes do not extend laterally beyond the lateral water-vascular vessels.'

In the specimens from Kingston, about 40 testes, only, are present in each segment, but they are very large and would accordingly be present in many consecutive sections.

There can be little doubt that the genera *Linstowia*, Zschokke, 1898, and *Oochoristica*, Lühe, 1898, are very closely related, if not identical.

Family *DAVAINEIDAE*, Fuhr., 1907.

Sub-family *DAVAINEINAE*, Braun, 1900.

Davainea microscolecina, Fuhrmann, 1909. Several specimens of this species collected and presented by Professor Warrington Yorke, M.D., Nawalia, North-eastern Rhodesia. June 28, 1911.

Host :—An unknown bird, the vernacular name of which is *Nduwaruwo*.

Family *HYMENOLEPIDIDAE*, Railliet and Henry, 1909.

Sub-family *HYMENOLEPIDINAE*, Ransom, 1909.

Hymenolepis interruptus, Clerc, 1906. Two specimens from a sparrow *Passer domesticus*, Hoylake, Cheshire, England. Collected and presented by Professor J. W. W. Stephens, F.R.S.

Echinocotyle nitidulans (Krabbe, 1882), Fuhrmann, 1906 (figs. 1-3). Numerous specimens from the intestine of *Tringa alpina*, Hoylake, Cheshire, England.

As the anatomy of this worm is not known, the following details are added.

The worms measure about 7 mm. in length and the maximum breadth is about 140μ; the number of segments varies from about 70 to 112, the average number in nine worms being 93. The segments are imbricated, and the last ones are almost square and measure about 100μ. The head measures about 130μ in length and 145μ in breadth. The rostellum is very prominent and measures about 75μ in length, terminating anteriorly in an expansion. It can be withdrawn into a very deep muscular sac which is dilated and extends into the neck region. It is armed with a single crown of ten simple hooks which measure 55μ. The neck measures about 380μ in length. There are three testes all in a line, and situated posteriorly.

FIG. 1. *Echinocotyle nitidulans.* Head. × 160.

The cirrus pouch is enormous and very muscular; it extends practically across the entire segment and has a breadth of about 25μ. No spines could be seen on the cirrus even under an oil immersion lens. The vas deferens on leaving the cirrus pouch, runs parallel, and posterior to the pouch, dilating into a seminal vesicle. Immediately median to this vesicle a prominent prostate gland can be seen enveloping a short portion of the vas deferens; it appears to be chitinous and resembles a spine; this portion of the vas deferens is very striking under high power magnifications. The vas deferens then splits up into the vasa efferentia.

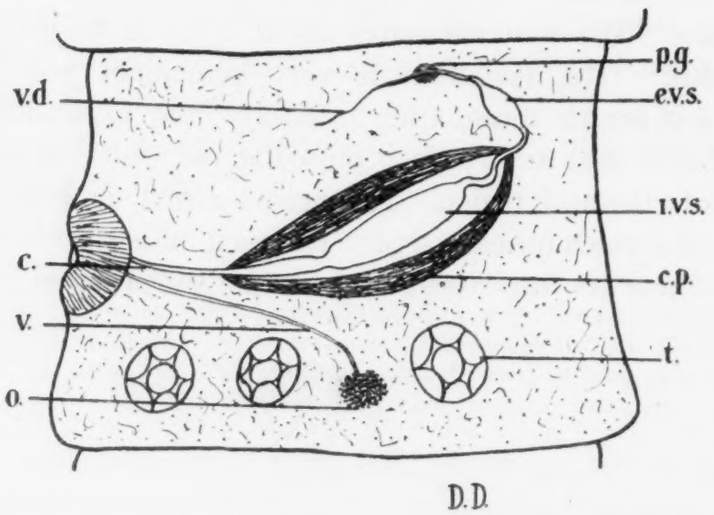

FIG. 2. *Echinocotyle nitidulans.* Segment nearly ripe. *v.d.*—vas deferens; *c*—cirrus; *v*—vagina; *o*—ovary; *t*—testes; *c.p.*—cirrus pouch; *i.v.s.*—internal seminal vesicle; *e.v.s.*—external seminal vesicle; *p.g.*—prostate gland. × 320.

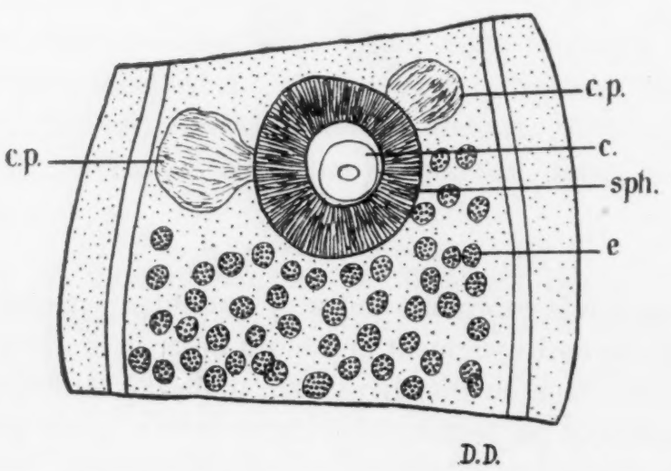

FIG. 3. *Echinocotyle nitidulans.* Side view of gravid segment showing pore. *c.p.*—cirrus pouch; *e*—eggs; *sph.*—sphincter muscle; *c*—cirrus. × 320.

The genital pores are unilateral and are surrounded by a very powerful sphincter muscle.

The uterus is a simple sac; in many segments it was full of immature eggs. No ripe eggs were seen.

There appears to be no reason for separating this genus (which contains eight species only) from the genus *Hymenolepis*.

Family *TAENIIDAE*, Ludwig, 1886.
Sub-family *TAENIINAE*, Stiles, 1886.

Multiceps serialis (Gervais, 1847), Stiles and Stevenson, 1905. Four specimens from a Hyena (spotted leopard), Ngoa, North-eastern Rhodesia. Collected and presented by Professor Warrington Yorke, M.D.

The worms measured about 45 cms. in length and the last segments about 1·3 cms. in length.—Genital pores large, and situated behind the middle of the lateral margin.

Head with a double crown of hooks, the larger measuring 160 μ in length and the smaller 85 μ in length. Uterus with from 14 to 21 lateral branches.

This parasite has hitherto been recorded from *Canis familiaris*, *Lepus caniculus* and *Sciurus carolinensis*.

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A SURVEY OF THE PARASITES FOUND IN NATIVES OF SIERRA LEONE

BY

P. A. MAPLESTONE

(*From the Sir Alfred Jones Research Laboratory, Freetown, Sierra Leone*)

(*Received for publication 2 May*, 1924)

This investigation was carried out with the object of obtaining an indication of the infection rate among Sierra Leone natives of the common intestinal parasites.

The stools of five hundred natives, all prisoners in the Freetown gaol and all adult males, were examined. The results are, therefore, not quite representative of the native population as a whole, as women and children are not included. Nevertheless, so far as it goes, it is fairly comprehensive, because all the tribes found in Sierra Leone Protectorate are represented and 75 per cent. of the people examined were living in Freetown at the time of arrest; the remaining 25 per cent. were living in various parts of the Protectorate. Gaol infection may be excluded, for the examinations were made within a few days of incarceration in the prison.

Only a single specimen of faeces was examined from each individual, but at least two smears in normal saline under a seven-eighths inch square cover glass were completely examined in every case, and where necessary, specimens in Lugol's solution were made as well. In addition to this examination, a portion of the faeces was mixed in saturated salt solution for detection of helminth eggs that had been missed in the thin smears.

(*a*) Protozoa

Table I gives a list of all the Protozoa encountered, with the number of infections expressed in percentages.

Table I

Parasite	Per cent. infected	Parasite	Per cent. infected
Entamoeba histolytica	15	*Giardia intestinalis*	2·2
,, *coli*	43·6	*Chilomastix mesnili*	1·8
Endolimax nana	9·8	*Trichomonas hominis*	2·2
Iodamoeba butschlii	13	*Enteromonas intestinalis*	2·4
		Coprozoic flagellates	14·6

The nomenclature in the above table is taken from Dobell and O'Connor (1921), and under 'Coprozoic flagellates' are grouped all the species so classified by the same authors, because their cysts are not distinguishable from each other in fresh smears.

There is nothing remarkable in the above figures, except perhaps the small number of natives infected with certain flagellates, especially *G. intestinalis*. Vegetative forms of the Amoebae and their cysts have been included under the one head. Five cases of infection of Amoebae with *Sphaerita* spp. were encountered; two of these were in *E. histolytica*, two in *E. coli* and one in *I. butschlii*.

There were, of course, several cases of infection with two or more parasites, but no analysis of these has been made, for it is not considered that such figures are of any practical importance.

(*b*) Helminths.

In Table II are given a list of the intestinal worms found, the diagnosis being made by the presence of eggs in the faeces. The infection rate is expressed in percentages of the 500 stools examined.

TABLE II

Parasite	Per cent. infected	Parasite	Per cent. infected
Ascaris lumbricoides	36·6	*Enterobius vermicularis*	1·6
Trichuris trichiura...	18·6	Hookworm*	76·6
Strongyloides stercoralis	15·2	*Taenia* sp.	3·2

* Dr. Adler, of the Sir A. L. Jones Laboratory, examined 4,305 adult Hookworms taken from 47 different cases and found that 91·3 per cent. were *N. americanus* and 8·7 per cent. were *A. duodenale*. Of the total of 376 *A. duodenale*, 302 came from two cases who were not gaol prisoners. Worms examined from prisoners were *N. americanus* 98 per cent. and *A. duodenale* 2 per cent., which is probably the more correct figure for the Colony as a whole.

The percentage of *S. stercoralis* is probably much lower than the actual number, for many faeces, negative by ordinary examination, were found to contain this parasite after culturing which was being done for another purpose. Indeed, it is considered probable that if this method were employed, the infection rate with *S. stercoralis* would be found to be almost, if not quite, as high as that with Hookworm.

The *Taenia* cases are probably all *T. saginata*, for that is the only cestode the writer has found in natives in Freetown ; the eggs were slightly oval and none were round, this being given as a diagnostic character between the eggs of *T. saginata* and *T. solium*.

MICROFILARIA.

Thick films of night blood were examined in 288 cases and *Mf. bancrofti* were present in 16·3 per cent., and *Mf. perstans* in 2·4 per cent. Out of the 195 thick films of day blood no infections with *Mf. Loa* were discovered.

(*c*) A NOTE ON THE USE OF SATURATED SALT SOLUTION FOR THE DETECTION OF HELMINTH EGGS IN FAECES.

Lane (1922) has discussed this method in detail along with others, and he drew attention to its value for the detection of eggs other than those of hookworm, so it is only proposed to deal with the method quite briefly, in confirmation of Lane's remarks.

Technique :—

Small solid watch glasses were used for mixing the faeces; they contain about 8 c.c. when filled sufficiently to have a distinct convex meniscus. Care was taken to use a little less than 0·5 grams of faeces so as to keep within the limit of effective concentration; the importance of this precaution has been shown by Lane. It was found quite easy after a little practice to take the right amount of faeces by guesswork. The faeces were first thoroughly emulsified in about 2 c.c. of salt solution by stirring with a glass rod, more salt solution was then added, until there was a distinct convex meniscus above the surface of the watch glass. The emulsion was left for about five minutes to allow the eggs to come to the surface, and a glass slide 3 ins. by 2 ins. was laid on top of it. The slide was lifted off in about half a minute and rapidly turned over to bring the wet surface uppermost without allowing the fluid to run off, and it was examined under the low power of the microscope without a cover slip. The eggs, whatever the species, were always floating on the surface of the film of fluid, therefore even in the case of hookworm eggs, the character they possess of adhering to glass played no part in the success of the method.

The saturated salt solution was always prepared by boiling excess of salt in water and allowing it to cool, and as Lane (1922) has pointed out, this method of its preparation always assured the obtaining of a solution of slightly over 1,200 specific gravity.

Table III gives the number of times each species of egg was found in plain smears and the number of times it was found in salt solution after having been missed in plain smears; when eggs were found in plain smears, they were always found again by salt concentration.

TABLE III

	Ascaris		*Trichuris*		*Strongyloides*		*Oxyuris*		Hookworm		*Taenia*	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Plain smear ...	159	86·9	36	38·7	74	97·4	6	75	223	58·2	15	88·2
Salt solution ...	24	13·1	57	61·3	2	2·6	2	25	160	41·8	2	11·8

The different percentages of positive results for different eggs is due to the number present in a given volume of faeces; those

species such as *Ascaris*, in which a large number of eggs are present give a relatively high number of positives in plain smears and a correspondingly low number after salt concentration, whereas in the case of *Trichuris* which only has few eggs present, the reverse is the case. As Lane has already found, the larvae of *Strongyloides* do not readily come to the surface in salt solution, in fact, many cases which were positive in plain smears were negative in salt concentration.

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A CRITICAL EXAMINATION OF STOLL'S METHOD OF COUNTING HOOKWORM EGGS IN FAECES

BY

P. A. MAPLESTONE

(From the Sir Alfred Jones Research Laboratory, Freetown, Sierra Leone)

(Received for publication 2 May, 1924)

Stoll (1923) published a method of estimating the number of hookworm eggs in faeces. The evidence he adduced of the accuracy of his method may be summarised as follows :—

(1) No cultures of the same faeces produced as many larvae as were shown by egg counts.

(2) Soft stools gave less eggs than solid faeces from the same individual.

(3) The first portion passed of a formed stool gave higher counts than random samples of the whole of the same stool. This is explained by the fact that the first portion of a formed stool is drier and hence the eggs are more concentrated in that portion than in later and softer parts of the same stool.

(4) Random samples from the last and softer part of a stool gave slightly lower egg counts than the whole stool mixed.

(5) Counts from day to day from a single individual remained uniform, and this also applied to counts of *Ascaris*, *Trichuris*, and *Schistosomum* eggs.

(6) Counts from two distinct samples of the same stool are always fairly close together.

Stoll's evidence seems conclusive, but it is at best only circumstantial and the writer found it frequently inaccurate in one particular, viz. : that control cultures always produced fewer larvae than the number of eggs counted in the same faeces. This led to examining the method more closely and testing it in various ways.

Faeces were taken and counts performed in exactly the same way as Stoll describes, and, in addition, small portions (about 200 milligrammes) of the same faeces were accurately weighed in small solid watch glasses and thoroughly mixed with saturated salt solution. Slide after slide was then applied to the surface of the fluid in the watch glasses and all the eggs so obtained counted. This was continued until it was thought that nearly all the eggs had been removed.

SPECIMEN 1.

Counted by Stoll's method. 1st Count, 8. 2nd Count, 10. No. of eggs per gram of faeces average 900.

219 mgr. of faeces mixed with saturated salt solution gave 369+333+162+76 +103+7 eggs on six successive slides.

Total 1,050, which = 4,794 eggs per gram.

202 mgr. of faeces mixed with sat. salt sol. gave 352 + 274 + 251 + 148 + 66 eggs on five successive slides.

Total 1,091, which = 5,391 eggs per gram.

The average number of eggs per gram of two counts by salt concentration was 5,092.

Control cultures produced an average of 4,080 larvae per gram.

SPECIMEN 2.

Counted by Stoll's method. 1st Count, 6. 2nd Count, 6. No. of eggs per gram of faeces average 600.

201 mgr. of faeces mixed with sat. salt sol. gave 9 + 19 + 16 + 11 eggs on four successive slides.

Total 50, which = 274 eggs per gram.

201 mgr. of faeces mixed with sat. salt sol. gave 15 + 9 + 14 + 2 eggs on four successive slides.

Total 40, which = 199 eggs per gram.

The average number of eggs per gram of two counts by salt concentration was 236.5.

Control cultures were not done.

SPECIMEN 3.

Counted by Stoll's method. 1st Count, 2. 2nd Count, 1.

No. of eggs per gram of faeces average 150.

201 mgr. of faeces mixed with sat. salt sol. gave 8 + 9 + 11 + 6 + 10 + 3 eggs on six successive slides.

Total 47, which = 233 eggs per gram.

200 mgr. of faeces mixed with sat. salt sol. gave 13 + 13 + 5 + 9 + 3 + 3 eggs on six successive slides.

Total 46, which = 230 eggs per gram.

The average number of eggs per gram of two counts by salt concentration was 231.5.

Control cultures produced an average of 353 larvae per gram.

SPECIMEN 4.

Counted by Stoll's method. 1st Count, 2. 2nd Count, 1.

No. of eggs per gram of faeces average 150.

200.5 mgr. of faeces mixed with sat. salt sol. gave 8 + 35 + 21 + 7 + 3 eggs on five successive slides.

Total 74, which = 369 eggs per gram.

200.5 mgr. of faeces mixed with sat. salt sol. gave 18 + 15 + 32 + 13 + 8 eggs on five successive slides.

Total 86, which = 429 eggs per gram.

The average number of eggs per gram of two counts by salt concentration was 369.

Control cultures produced an average of 450 larvae per gram.

SPECIMEN 5.

Counted by Stoll's method. 1st Count, 6. 2nd Count, 6.

No. of eggs per gram of faeces average 600.

207 mgr. of faeces mixed with sat. salt sol. gave 27 + 52 + 3 + 2 + 0 on five successive slides.

Total 84, which = 406 eggs per gram.

201 mgr. of faeces mixed with sat. salt sol. gave 24 + 35 + 16 + 2 + 0 on five successive slides.

Total 77, which = 383 eggs per gram.

The average number of eggs per gram of two counts by salt concentration was 394.5.

Control cultures produced an average of 796 larvae per gram.

SPECIMEN 6.

Counted by Stoll's method. 1st Count, 100. 2nd Count, 100.

No. of eggs per gram of faeces average 10,000.

210 mgr. of faeces mixed with sat. salt sol. gave 323 + 319 + 471 + 162 + 72 on five successive slides.

Total 1,347, which = 6,414 eggs per gram.

Control cultures produced an average of 8,833 larvae per gram.

Stoll's method was further checked in the following manner :— Two counts were made by Stoll's method, his instructions being followed in every detail. About ten grams of faeces were placed in a beaker and balanced against an equal weight of water, the two being thoroughly mixed by stirring until the mixture was perfectly homogeneous. Three-gram lots of this mixture were now weighed out

and counted by Stoll's method. It was previously found that faeces are practically of a specific gravity of 1, therefore when mixed with an equal weight of water, a given weight of the mixture will only contain half the amount of faeces that is contained in the same weight of undiluted faeces. In Stoll's method, using undiluted faeces, the number obtained by counting is multiplied by 100 to ascertain the number of eggs in a gram of faeces; accordingly, if the same weight of the diluted faeces is taken and counted by Stoll's method, it will be necessary to multiply the count by 200 to obtain

TABLE 1

SUMMARY OF ABOVE COUNTS

Number of specimen	Eggs per gram by Stoll's method	Eggs per gram by salt concentration	Larvae per gram in control cultures
1	900	5,092	4,080
2	600	236·5	Not done
3	150	231·5	353
4	150	369	450
5	600	394·5	796
6	10,000	6,414	8,833

the number of eggs per gram in the undiluted faeces, and if Stoll's method is correct these two results should be approximately the same. If the dilution is still more increased by taking part of the faeces and water mixture already made and mixing it with an equal weight of water, a count of this dilution, if counted by Stoll's method, will have to be multiplied by 400 to give the number of eggs per gram in the original faeces. Further dilution in the same proportions will require multiplication by 800.

The above process was carried out with several specimens of faeces and the results are given in Table II. In this table 'first dilution' means original faeces plus an equal weight of water, 'second dilution' means part of 'first dilution' plus an equal weight of water, and 'third dilution' means a part of 'second dilution' plus an equal weight of water.

Number of specimen	Character of Faeces	Undiluted Faec		
		1st Count	2nd Count	E p g
1	Soft, formed... ...	100	117	10,
1 ... ... (second count)		—	—	
2	Very watery... ...	23	17	2,
2 ... ... (second count)		21	20	2,
3	Soft, formed... ...	5	4	
4	Liquid	26	41	3,
5	Soft, formed... ...	53	65	5,
5 ... ... (second count)		56	65	6,
6	Watery, with mucus	9	6	
7	Formed	0	0	
8	Formed, a little softer than 7	1	1	
9	Soft, unformed ...	0	1	
10	Liquid	2	2	
11	Liquid	11	6	

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TABLE I

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4	150	369	450
5	600	394·5	796
6	10,000	6,414	8,833

the number of eggs per gram in the undiluted faeces, and if Stoll's method is correct these two results should be approximately the same. If the dilution is still more increased by taking part of the faeces and water mixture already made and mixing it with an equal weight of water, a count of this dilution, if counted by Stoll's method, will have to be multiplied by 400 to give the number of eggs per gram in the original faeces. Further dilution in the same proportions will require multiplication by 800.

The above process was carried out with several specimens of faeces and the results are given in Table II. In this table 'first dilution' means original faeces plus an equal weight of water, 'second dilution' means part of 'first dilution' plus an equal weight of water, and 'third dilution' means a part of 'second dilution' plus an equal weight of water.

TABLE II

Number of specimen	Character of Faeces	Undiluted Faeces			First Dilution			Second Dilution			Third Dilution		
		1st Count	2nd Count	Eggs per gram	1st Count	2nd Count	Eggs per gram	1st Count	2nd Count	Eggs per gram	1st Count	2nd Count	Eggs per gram
1	Soft, formed... ...	100	117	10,850	69	76	14,500	51	41	18,400	—	—	—
1 ... ... (second count)		—	—	—	78	72	15,000	—	—	—	—	—	—
2	Very watery... ...	23	17	2,000	9	8	1,700	—	—	—	—	—	—
2 ... ... (second count)		21	20	2,050	12	12	2,400	—	—	—	—	—	—
3	Soft, formed... ...	5	4	450	8	4	1,200	2	2	800	—	—	—
4	Liquid	26	41	3,350	15	22	3,700	12	9	4,200	—	—	—
5	Soft, formed... ...	53	65	5,900	95	102	19,700	56	64	24,000	—	—	—
5 ... ... (second count)		56	65	6,050	86	95	18,100	41	50	18,200	—	—	—
6	Watery, with mucus	9	6	750	3	0	300	1	0	200	—	—	—
7	Formed	0	0	0	1	1	200	0	1	200	—	—	—
8	Formed, a little softer than 7	1	1	100	0	3	300	0	3	600	0	0	0
9	Soft, unformed ...	0	1	50	0	1	100	1	1	400	—	—	—
10	Liquid	2	2	200	2	4	600	3	2	1,000	2	1	1,200
11	Liquid	11	6	850	7	3	1,000	0	2	400	3	2	2,000

All the weighings and counts were done personally by the writer with the greatest possible care, and accuracy was in no case sacrificed on account of the time taken ; slides were counted twice if there was any doubt that eggs had been missed or counted more than once owing to inaccurate moving of the mechanical stage of the microscope.

The above results obtained by checking Stoll's method against salt concentration and dilution of faeces indicate that the method is not accurate within ten per cent., which is the claim of the originator.

Examination of Table II suggests that the number of eggs obtained by this method varies with the consistency of the original specimen of faeces, higher counts being obtained when the faeces are liquid. The reason for this is difficult to explain, for shaking with glass beads in a solution of deci-normal NaOH for a minute or more, as recommended by Stoll, seems to cause just as complete comminution of the faeces whether they are solid, soft, or liquid to begin with.

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ON THE LIFE HISTORY OF A REPTILIAN TAPEWORM *(SPARGANUM REPTANS)*

BY

F. J. MEGGITT, M.Sc., PH.D.

PROFESSOR OF BIOLOGY, UNIVERSITY OF RANGOON

(*Received for publication*, 19 *May*, 1924)

PLATE IX

The genus *Sparganum* was first proposed by Diesing (1854, 573) for those Pseudophyllidean cestodes the adult stages of which were unknown, and has included at various times *S. affine*, Diesing, 1854, *S. ardeae-coeruleae* (Diesing, 1850), *S. baxteri*, Sambon, 1907, *S. ellipticum*, Molin, 1858, *S. erinacei-europaei* (Rudolphi, 1819), *S. falconis* (Rudolphi, 1819), *S. lanceolatum*, Molin, 1859, *S. lanii-pomerani* (Rudolphi, 1819), *S. mansoni* (Cobbold, 1883), *S. mygales-moschatae* (Rudolphi, 1819), *S. proliferum* (Ijima, 1905), *S. raillieti*, Rátz, 1913, *S. reptans* (Diesing, 1850), *S. sebago*, Ward, 1910, *S. strigis-accipitrinae* (Rudolphi, 1819). Most of these are probably synonyms, but the absence of diagnostic characters renders identification impossible except by feeding experiments. Of the above fifteen forms, only the life-histories of *S. mansoni* and *S. raillieti* have been elucidated.

S. reptans is essentially parasitic in reptiles, occurring in the connective tissue and between the muscles—usually dorsal—of *Amphisbaena flavescens**, *Anabates lucinioides*, *Drymobius bifossatus* (Raddi, 1820), *Elaps marcgravii*, Wied, 1820, *Erythrolampus aesculapii* (L.1754) *Herpetodryas carinata* (L.1754), *Lachesis lanceolatus* (Jonnès, 1816), *L. neuwiedii* (Wagl. 1824), *Leptophis liocerus* (Wied, 1824) *Oxyrhopus cloelia* (Daud, 1803), *Pseudophis*

* In consequence of the scanty literature at my disposal, it has proved impossible to check all the host names.

bivittatus, P. cinerascens and *Xenodon merremii* (Wagl. 1824). It has also been recorded from :—

AMPHIBIA : *Alcedo americana, Hydroscopus plumbeus, Lyophis regius, Rhynaspis proboscidea, Spilotes pullatus.*

AVES : *Ardea coccoi, A. leuce, A. lineata, Corvus azureus, C. cristalellatus, C. cyanomelas, C. pileatus, Ibis albicollis, Merganser brasiliensis* (Vieill.), *Merula albiventer*, Spix, *M. rufiventris*, Vieill., *Molothrus ater* (Bodd.), *Musicapa psalura, Nonnula rubecula* (Spix), *Nothocrax urumutum* (Spix), *Ostinops decumanus* (Pallas), *Pandion haliaëtus* (L.) *Rhamphastos temminckii R. toco* (Müller), *Rhynchotus rufescens* (Temm.), *Strix grallaria, Tantalus loculator* (L.).

MAMMALIA : *Canis azarae*, Wied, 1826, *Chloroceryle americana, Chrysothrix sciurea* (L.1766), *Didelphys brachyura, D. opossum*, Seba, 1734, *Felis mitis*, Cuvier, 1820, *F. pardus*, L.1766, *F. tigrina*, Erxleben, 1777, *Galera barbara* (L.1766), *Gulo barbatus, Hapale melanura* (Geoffroy, 1812), *Holochilus brasiliensis* (Geoffroy, 1819), *Lutra brasiliensis*, Zimmermann, 1780, *L. paranensis*, Rengg, 1830, *Nasua narica* (L.1766), *Noctilio leporinus*, L.1766, *Saïmiris sciurea* (L.1766).

All records, however, other than those from reptiles, should be regarded with suspicion, the absence of distinguishing characters rendering it probable that several species have been confused under one name.

S. reptans is an exceedingly common parasite in Burmese snakes. From one *Tropidonotus*, sp., twenty specimens were obtained, from another two, and from four more approximately fifteen each. With this material an attempt was made to discover the life-history. On January 20th, ten active specimens were given to a puppy a month old, and six to a human volunteer. On February 20th a further ten were given to the dog, who in the meantime had grown and fattened. He was killed on March 3rd, and was found to contain three adult *Dipyllidium caninum*, three full-grown *Diphyllobothrium* and two scoleces of the same genus. The experiment is not conclusive as no time was available for previous faecal examinations of the animal nor for treatment with anthelminthics. Considering the youth of the dog and the absence of any records of Pseudophyllidea from Indian dogs, it is exceedingly improbable that the

Diphyllobothrium were present previous to the experiment. The presence of the worms produced in the animal no symptoms whatever. No trace of infection has been found in the faecal examinations of the human subject with the exception of a single egg, probably due to a contaminated slide. Attempts were made to ascertain if the life-history conforms to the type of *D. latum* and *D. mansoni* described by Rosen (1918) and Okomura (1919) respectively, but up to the present no procercoids have been found in the experimental Entomostraca.

PLEROCERCOID STAGE

The plerocercoid stage is usually to be found in small sacs on each side of the vertebral column between the skin and musculature of the dorsal surface, also amongst the connective tissue. It is motionless *in situ*, but upon being deposited in warm water becomes exceedingly active, wriggling in a manner reminiscent of a nematode. The scolex in particular performs active and regular movements. The tip is first protruded like a small tongue, becomes flattened while the sides swell up to form a truncated square pyramid, and is then retracted again, leaving a small pit at the apex of the pyramid. The whole anterior extremity subsides immediately, to repeat the same movements without pause. There is no definite scolex nor bothridia, only the mobile anterior extremity. The alleged terminal invagination is the result of contraction consequent upon fixation and, while probably performing the functions, has no trace of the structure of a sucker. External and internal segmentation is usually absent, but may occasionally in unusually long forms be represented by a few posterior transverse striations. The body is a slender ribbon with an anterior globular swelling, varying in length from 2 to 100 mm. and is capable of asexual reproduction by fragmentation but not by proliferation. In several cases strobilae were observed which were obviously the result of fragmentation and which were leading an independent existence. The internal anatomy showed nothing of note except the absence of 'nutritive bodies' described by Ijima (1905, 15) and myself (1924, 53) for *S. prolifer* and *Sparganum*, sp. respectively. In the absence of other distinctions it is possible that this character may be of use in identification.

ADULT FORM

Length 1000 mm. by 9 mm. wide, clearly segmented externally though with but little overlapping of the proglottides. Scolex (Pl. IX, fig. 1) elongated, 800μ long by 40μ wide, bearing two long shallow bothridia with indistinct edges, merging anteriorly and posteriorly into the scolex. Neck elongated. All segments broader than long. Musculature weak, consisting of a narrow and feeble layer of longitudinal muscles : transverse muscles could not be seen. Excretory vessels indistinct, consisting of four to eight longitudinal trunks on each side of the proglottis, connected by an extensive and complicated capillary system. Genital pores (Pl. IX, fig. 2) superficial, on same surface of proglottis. Male in anterior sixth, central : vaginal posterior to it and slightly lateral : uterine more posterior and central. Cirrus-sac extending half-way to opposite surface, external vesicula seminalis nearly reaching aporal cortical parenchyma. Testes in transverse sections 8 to 10 each side, in longitudinal vertical ones 9 to 11, total 144 to 220 : in two separate lateral bands, slightly converging anteriorly. Ovary bi-lobed, reticulate. Shell-gland large, at posterior margin of proglottis. Vitelline glands lateral, converging and meeting anteriorly, and leaving free a central space one-twelfth to one-seventh of width of proglottis.

Eggs 53μ to 59μ by 36μ to 40μ, operculated : immature when deposited, onchosphere develops while egg lies in water.

From the characters just given, it may be seen that the adult of *S. reptans* belongs to the genus *Diphyllobothrium*. Altogether forty-seven species of this genus have been recorded, but the descriptions of only twenty-eight contain characters of diagnostic value, the remainder being concerned only with length and breadth of scolex and strobilus, shape of proglottides, etc.

An examination of the following table giving the species of this genus, indicates that well-marked features (distribution of testes, position and number of uterine coils, etc.) separate *D. reptans* from the majority of forms. From the remainder many may be separated by the size of the egg, a character liable to variation but only within fairly definite limits. *D. exile* and *D. fissiceps* have only been recorded once and are dubious species, while *D. nasuta* according to Fuhrmann

(1908, 95) should no longer be recognised. Minor differences separate *D. stemmacephalum* (shape of bothridia), *D. folium* (absence of neck and shape of proglottides, *D. rufum* (pigmentation of scolex) and *D. americanum* (absence of neck). These features are of slight value in themselves, but constitute the only distinguishing marks of the species. From *D. strigis-accipitrinae, D. decipiens, D. sulcatum, D. similis,* and *D. marginatus* the present form cannot be separated at all, owing to the absence of any description. The description of *D. clavatum* is not accessible here. It may, therefore, be concluded with reasonable accuracy, that *D. reptans* is a distinct species. The adult host remains to be discovered. The dog is obviously only an experimental host, the true one is probably a carnivore or an avian scavenger.

Specimens of adult and larval forms have been deposited in the Molteno Institute for Parasitology, Cambridge.

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Species		Genital pore	Uterine pore	Cirrus-sac	Testes	Uterine coils	Enlargement of uterus	Eggs (in μ)	Host
D. americanum ...	Hall and Wigdor, 1918	...	...	...	...	...	...	...	*Canis familiaris*
D. archeri	(Leiper and Atkinson, 1914)	Half-way	Median	5 lobed internally	Scattered	...	...	...	*Ogmorhinus weddelli*
D. canadense ...	Cooper, 1921	Anterior third, vaginal lateral	Median	...	150, 2 lateral fields joined anteriorly	8 to 10 each side	Absent, coils break down into sac	56 to 59 by 37 to 39	*Corvus corax principalis*
D. clavatum ...	Railliet and Henry 1912	...	...	...	...	...	...	...	*Ogmorhinus weddelli*
D. coatsi	(Rennie and Reid, 1912)	...	Median	...	90, scattered	Absent, contain few eggs	...	60	*Ogmorhinus weddelli*
D. coniceps	(Linstow, 1905)	Anterior	Median	Extends half-way between surfaces	...	...	Present	...	*Phoca barbata*
D. cordatum ...	(Leuckart, 1863)	...	Median	...	240 to 300, 2 separate lateral bands	6 to 8, extending laterally to genital pore	...	70 to 80 by 50	*Canis familiaris, Homo sapiens, Phoca barbata, P. greenlandica Trichechus rosmarus*
D. cordiceps... ...	(Leidy, 1872)	...	...	...	...	...	...	70 by 35	*Pelecanus erythrorbynchus*
D. decipiens ...	(Diesing, 1850)	...	...	Extends half-way between surfaces	...	...	...	50 by 60	*Canis familiaris, C. lupus, Felis cattus, F. concolor, F. domestica, F. macroura, F. mellivora, F. mitis, F. onca, F. pardus, F. tigrina, mellivora ratel*
D. dendriticum ...	(Nitzsch, 1824)	...	...	...	470, 2 lateral fields joined anteriorly	8 to 9	...	...	*Larus argentatus, L. canis, L. ridibundus, Rissa tridactyla*
D. ditremum ...	(Creplin, 1825)	...	...	...	380 to 390, 2 lateral fields joined posteriorly and anteriorly	7	...	...	*Larus argentatus, Mergus merganser, M. serrator, Urinator arcticus, V. stellutus*

Species		Genital pore	Uterine pore	Cirrus-sac	Testes	Uterine coils	Enlargement of uterus	Eggs (in μ)	Host
D. elegans	(Krabbe, 1865)	...	...	...	...	...	...	44 by 35	*Cystophora cristata, Eumetopias jubata, Phoca vitulina*
D. exile	(Linton, 1892)	...	...	...	...	...	...	...	*Larus californicus*
D. fissiceps	(Creplin, 1829)	...	...	...	...	...	...	...	*Sterna hirundo*
D. folium	(Diesing, 1850)	...	...	...	...	...	...	...	*Herpestes albicaudus*
D. fuscum	(Krabbe, 1865)	...	...	...	...	5 to 7 (Railliet 10 to 12), lateral to male genital pore	...	55 to 60, non-operculated	*Canis familiaris*
D. bians	(Diesing, 1850)	Cirrus posterior to vagina (Ariola)	...	...	...	...	...	59 by 38	*Monachus albiventer, Phoca barbata, P. hispida, P. vitulina*
D. lanceolatum ...	(Krabbe, 1865)	Anterior quarter	...	...	180 to 312, 2 separate fields	5 to 7, lateral to male genital pore	...	55 to 60 (Zschokke and Heitz 62 by 40)	*Phoca barbata*
D. lashleyi	(Leiper and Atkinson, 1914)	Anterior	Lateral	...	2 separate lateral bands with 2 posterior ones	Simple	...	60	*Ogmorhinus weddelli*
D. latum	(L.1758)	Anterior quarter	Median	Extends half-way between surfaces	2 separate lateral bands	Numerous, extending laterally to genital pore	Absent	67 to 70 by 48 to 54	*Canis azarae, C. familiaris, Cystophora cristata, Felis concolor, F. domestica, F. hernandesii, F. macroura, F. mellivora, F. mitis, F. pardus, F. tigrina, Herpestes leucurus, Homo sapiens, Leptonyx monachus, Odobaenus rosmarus, Phoca barbata, P. hispida, P. vitulina, Phocaena phocaena, Ursus maritimus, Vulpes lagopus, V. vulpes*

Species		Genital pore	Uterine pore	Cirrus-sac	Testes	Uterine coils	Enlargement of uterus	Eggs (in μ)	Host
D. macrophallus ...	(Linstow, 1905)	...	...	...	...	...	...	...	*Otaria ursina, Phoca barbata*
D. mansoni	(Cobbold, 1882)	...	...	...	...	...	...	63 to 76 by 31 to 43	*Canis familiaris*
D. marginatus ...	(Krefft, 1871)	...	...	...	...	...	...	...	*Halmaturus*, sp.
D. mobilis	(Rennie and Reid, 1912)	Anterior	Lateral	...	2 bands joining anteriorly	3 to 6	...	Fuhrmann 56 to 60 by 44 (Rennie and Reid 51 by 34)	*Ogmorhinus weddelli*
D. nasuta	(Rudolphi, 1802)	...	...	...	...	...	...	...	*Parus major*
D. parvum	(Stephens, 1908)	Like *D. latum*	...	...	...	...	...	...	*Homo sapiens*
D. perfoliatum ...	(Railliet and Henry, 1912)	...	Lateral	Extends half-way between surfaces	...	Only one coil with eggs	...	Fuhrmann 60 to 64 by 45 to 48 (Railliet and Henry 56 to 64 by 43 to 45)	*Ogmorhinus weddelli*
D. polycalceollum ...	(Ariola, 1896)	Anterior sixth	...	...	...	8 to 9, not lateral to genital pore	...	48 by 32	*Phoca vitulina*
D. podicipedis ...	(Diesing, 1854)	...	...	...	...	...	...	...	*Podiceps minor, P. rubricollis*
D. pygoscelis ...	(Rennie and Reid, 1912)	Very anterior	...	...	Scattered over proglottis	6 to 7	...	64 to 80 by 50 to 52	*Pygoscelis*, sp.
D. quadratum ...	(Linstow, 1891)	Anterior quarter	Median	Extends half-way between surfaces	...	6 to 7	Present	54 by 44 (Fuhrmann 50 by 43)	*Ogmorhinus leptonyx*
D. raillieti	(Rátz, 1913)	...	Median	Small	300 to 500, 2 separate fields	6 to 7	Present	62 to 70 by 37 to 54	*Canis familiaris*
D. resimum ...	Railliet and Henry 1912	...	...	...	...	...	...	...	*Ogmorhinus leptonyx*
D. römeri	(Zschokke, 1903)	Anterior fifth	Median	To aporal surface	600 to 1000	6 to 8, lateral to genital pore	...	62 by 39	*Trichechus rosmarus*

Species		Genital pore	Uterine pore	Cirrus-sac	Testes	Uterine coils	Enlargement of uterus	Eggs (in μ)	Host
D. rufum	(Leiper and Atkinson, 1914)	...	...	...	...	...	...	25	*Ogmorhinus weddelli*
D. schistochilos ...	(Germanos, 1895)	...	...	...	...	...	...	50 to 70 by 20 to 30	*Phoca barbata*
D. scotti	(Shipley, 1907)	...	Lateral	...	...	4	Present	Shipley 40 by 30, Fuhrmann 64 by 40	*Ommatophoca rossi*
D. scoticum ...	(Rennie and Reid, 1912)	...	Lateral	...	...	5 to 6	...	70 to 80 by 44 to 48	*Ogmorhinus leptonyx*
D. semiligula ...	(Nitzsch, 1824)	...	...	...	...	...	...	...	*Podiceps rubricollis*
D. serratum ...	(Diesing, 1850)	...	...	...	...	...	...	64 by 46	*Canis azarae, C. familiaris*
D. similis	(Krabbe, 1865)	...	...	...	...	...	...	60	*Vulpes lagopus*
D. spiraliceps ...	(Volz, 1900)	Uterine and genital pores one either side	...	...	...	...	...	36 by 32	*Falco concolor*
D. stemmacephalum	Cobbold, 1858	...	...	...	...	...	...	...	*Phocaena phocaena*
D. sulcatum ...	(Molin, 1858)	...	...	...	...	...	...	...	*Felis pardus*
D. tectum	(Linstow, 1892)	...	Lateral or median	To medullary parenchyma	...	...	...	65 by 47 (Fuhrmann 65 by 44)	*Macrorhinus leoninus*
D. wilsoni	(Shipley, 1907)	...	Lateral	Half-way between surfaces	...	...	...	(Fuhrmann 48 to 52), Rennie and Reid 52 to 60 by 32 to 36	*Ogmorhinus weddelli, Ommatophoca rossi*
D. reptans	(Diesing, 1854)	Anterior quarter	Median or lateral	Half-way between surfaces	144 to 220, 2 separate bands	4, not lateral to genital pore	Present	53 to 59 by 36 to 40	*Canis familiaris*

EXPLANATION OF PLATE IX

FIG. 1. *D. reptans :* scolex.

FIG. 2. *D. reptans :* mature proglottis. *c.s.*, cirrus-sac ; *m.*, male pore ; *ov.*, ovary ; *t.*, testes ; *u.*, uterus ; *v.*, vagina ; *v.*[1], vaginal pore ; *vit.*, vitellaria.

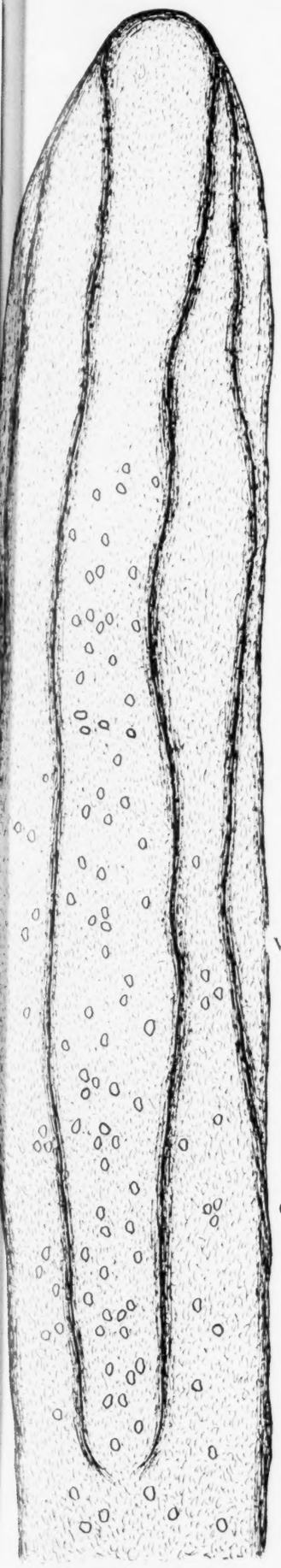

FIG. 1

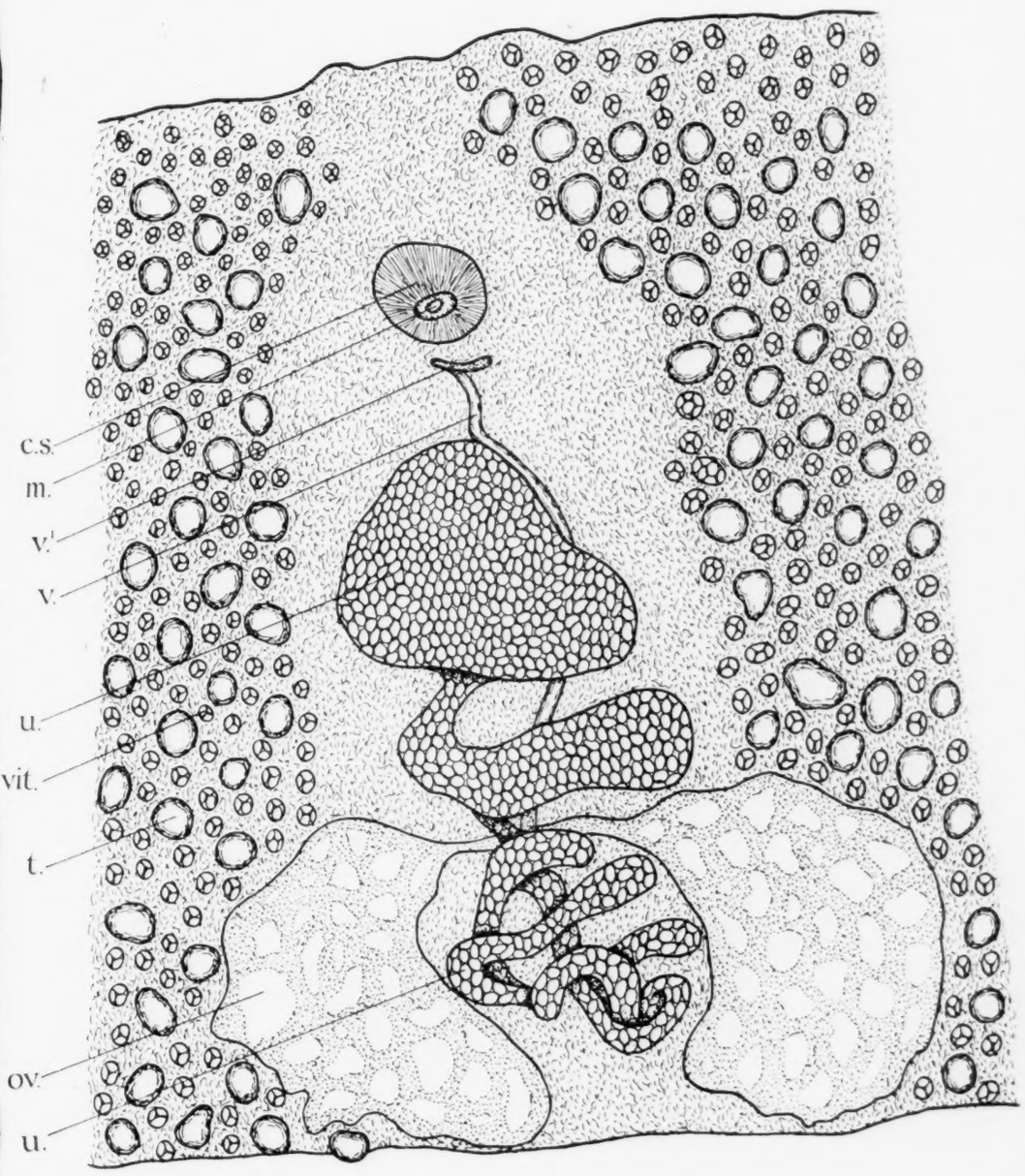

FIG. 2

C. Tinling & Co., Ltd., Imp.

A NEW VARIETY OF *GLOSSINA SCHWETZI*, NEWSTEAD & EVANS, FROM THE BELGIAN CONGO

BY

W. H. POTTS

(*Received for publication, 22 May,* 1924)

Glossina schwetzi var. *disjuncta* var. nov.

Colour and pattern as in typical G. SCHWETZI, *Newstead and Evans, but the distal processes of the harpes short and detached, and the median process more prominent.*

MALE. Length, 11·6 mm. ; length of palpi, 3·2 mm. ; width of head, 3·25 mm. ; length of wing, 10·6 mm.

Genital Armature (fig. 1, *A*). Differing from that of typical *G. schwetzi* in the following characters :—

(1) The *harpes* bears three pairs of processes (*h.*1 ; *h.*2 ; and *h.*3), the distal or third pair of which (*h.*3) is detached from the main body of the harpes, and, instead of being the longest, is barely as long as the second or middle pair (*h.*2).

(2) The *median process* (*m.p.*) is larger, and projects well beyond the inferior claspers (*i.c.*), between which it is situated.

An illustration of the harpes of *Glossina schwetzi* (fig. 1, *B*) has been added in order to facilitate the comparison of the two armatures.

The above description is based on one specimen, a male, in a collection sent to this School for identification by Dr. Schouteden from the Museum of the Belgian Congo, Tervueren. The fly is one of two accompanied by the following data :—' Sur buffle tué à Buku Kaie, 26.6.21, Rodhain.' The other fly proved to be a typical male *G. tabaniformis*.

The Type specimen is being sent back to the Museum of the Belgian Congo, Tervueren.

It is with great pleasure that I take this opportunity of thanking Professor Newstead, for his kind advice and assistance.

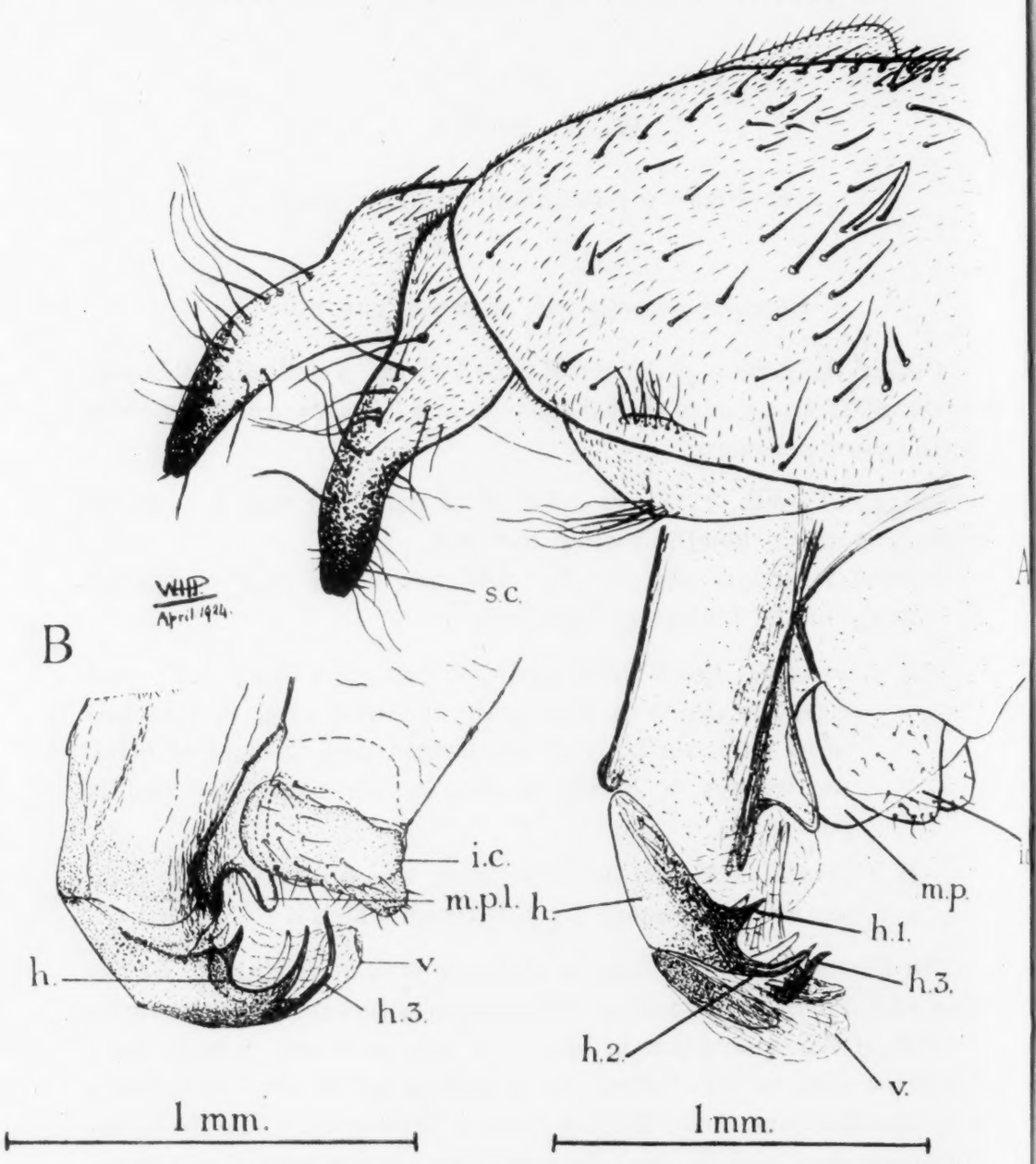

FIG. 1, *A*. Male Genital Armature of *Glossina schwetzi* var. *disjuncta* var. nov. (× about 60). *B*. Portion of male genital armature of *Glossina schwetzi*, Newstead and Evans (× about 50). *b*.—harpes; *b*.1, *b*.2, *b*.3—first, second, and third pairs of processes of the harpes; *i.c*.—inferior claspers; *m.p*.—median process; *m.p.l*.—inferior median process; *s.c*.—superior claspers; *v*.—vesica.

THE CRESCENT AND THE RED CELL

BY

J. W. W. STEPHENS

AND

R. M. GORDON

(*Received for publication 26 May*, 1924)

PLATES X-XIII

The following observations were made on a series of thin blood films from a case of malaria in the Federated Malay States. The films were made from 6.8.23 to 14.8.23, during which period the patient was taking quinine grains 20 daily.

Lawson (1911-1920) in a series of papers on the morphology of the malaria parasite, has published a number of beautiful photographs of crescents showing the variable relationship existing between the crescent and the red cell ; nevertheless, authors continue to publish illustrations representing practically only one stereotyped form.

In the present paper accordingly we have thought it worth while to draw attention to this variability.

We had also hoped to throw light on the vexed question as to whether the malaria parasite, in so far as the crescent is concerned, is in or on the red cell.

We may state at once that we consider that the specimens show clearly that in many cases the poles of the crescent project beyond the red cell, but we have been unable to determine the relationship of the body of the crescent to the red cell, although the general impression left on our minds after examining a very large number of crescents during the last three months is that the body also is extracellular.

Although we are unable to explain how the ' loop-form ' which is probably the commonest form, and which is the one almost

invariably shown in the text books, arises, yet we can trace a transition from the stage in which the red cell stains uniformly, having no portion decolorised, to the loop-form in which the red cell is entirely decolorised except for a skeleton outline or rim.

Lawson has already figured most if not all of the forms we propose to describe, but we think that a diagrammatic representation gives a clearer idea of the appearances actually seen, than does a photograph however beautifully executed.

It will be seen that the majority of the crescents figured are females. This is due to the fact that we frequently experienced difficulty in distinguishing the line of demarcation between the male crescent and the red cell as both stained an almost equally intense red.

This intense red staining of crescent-infected erythrocytes is peculiar and further they do not show any appearance resembling the stippling (Stephens' and Christophers' dots) characteristic of the ring forms of *Plasmodium falciparum.*

With regard to the crescent itself, it appears to us to be a flat ribbon-shaped structure, the ends of which, in thick portions of a film are generally incurved, while in thin portions the crescent lies flat.

We did not observe any forms that we could interpret as young or developmental forms.

The illustrations were made diagrammatically at a magnification of about 4,000 diameters. They may be arranged in four groups.

PLATE X (figs. 1-12)

Group A. Those in which the red cell stains uniformly, presenting a solid appearance.

Figs. 1, 2, 8, 9. The surface of the crescents appears to show buds or projections.

Fig. 6. Shows a projection at one pole. From its staining character it appeared to be red cell, but in many cases it was impossible to be certain as to the nature of these 'buds.'

Figs. 7-11. The poles of the crescent project beyond the red cell.

Fig. 12. Shows folding of the crescent.

PLATE XI (figs. 13-24)

Group B. Those in which the red cell is partly decolorised.

Figs. 13-18. More or less of the red cell is decolorised.

Figs. 19-24. A gradual transformation to the loop-form.

Fig. 23. Shows a solid wedge of non-decolorised red cell extending across the decolorised area.

Fig. 24. A somewhat similar appearance.

PLATE XII (figs. 25-36)

Group C. Those in which the greater part of the red cell is decolorised and in which part of the circumference of the red cell takes the form of a loop usually corrugated and generally subtending the concavity of the crescent.

Fig. 25. A typical loop-form. The crescent is completely surrounded by a rim of red cell, and the loop arises from two triangular thickenings on one side of it. These thickenings are situated not at the poles of the crescent but at some little distance therefrom. The rim of red cell presents coarse corrugations usually most prominent on its convexity, while the loop presents a series of finer corrugations. The loop bounds an area almost decolorised, but a granular basis can be frequently recognised.

Fig. 26. The crescent has pointed ends and projects beyond the red cell margin.

Fig. 27. A double-loop, a not uncommon form. The poles of the crescent are free.

Fig. 28. A similar form. One pole projects.

Figs. 29, 30. Two loops, both on the same side of the crescent. A possible interpretation is that the crescent lies between the folded red cell.

Figs. 31-35. Irregular loop formation.

Fig. 32. The outline of the red cell can be seen crossing the body of the crescent.

Fig. 36. Unusual position of the loop.

PLATE XIII (figs. 37-48)

Group D. Miscellaneous forms.

Figs. 37-45. The crescents are folded.

Figs. 37-39. The red cell is not decolorised.

Figs. 40-45. Various degrees of folding of the crescents and decolorisation of the red cell.

Fig. 46. A crescent with pointed poles (cp. fig. 26). These forms were encountered not infrequently. Their significance is unknown.

Figs. 47, 48. Double infection of the red cell. In both cases a male and a female crescent occurred in each red cell.

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PLATE X

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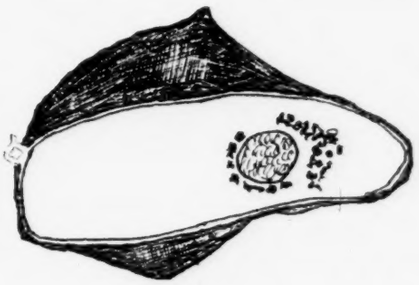

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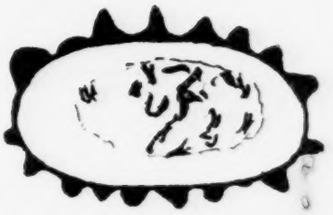

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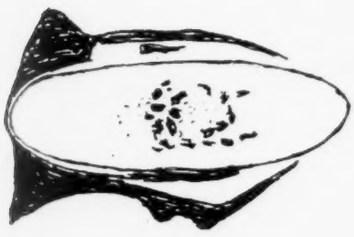

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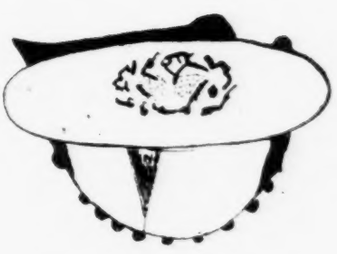

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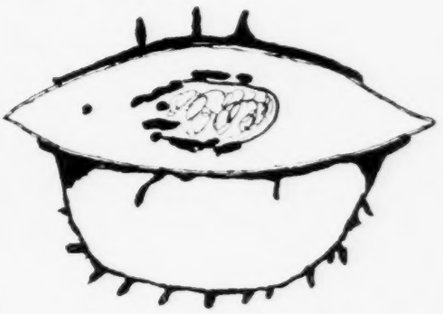

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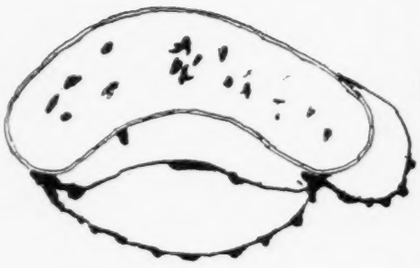

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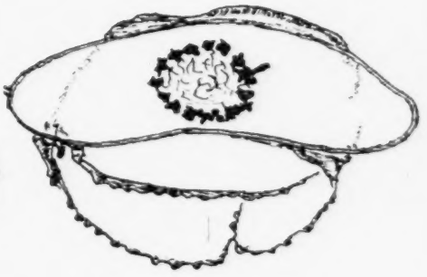

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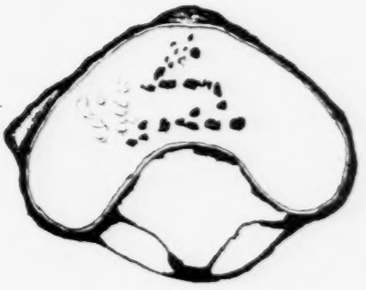

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PLATE XIII

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HUMAN SCHISTOSOMIASIS DUE TO *S. HAEMATOBIUM* IN SIERRA LEONE

BY

D. B. BLACKLOCK, M.D.

AND

M. G. THOMPSON, M.B., B.Sc.

(*From The Sir Alfred Lewis Jones Research Laboratory, Freetown, Sierra Leone*)

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PLATE XIV

During December, 1923, and January and February, 1924, an investigation was carried out into the prevalence of human Schistosomiasis in certain districts of the Protectorate of Sierra Leone. A Report on the subject has been submitted to the Government of Sierra Leone and will be published in the *Annual Medical and Sanitary Report* of the Colony.

The Report dealt with :

1. The prevalence of Urinary Schistosomiasis in the various districts traversed, and among the various tribes examined.
2. The discovery of the intermediate snail host ; this was proved to be *Physopsis* c.f. *globosa*, Morelet.
3. The rarity of snails of the genus *Planorbis* and the absence of Intestinal Schistosomiasis.
4. Tribal customs which facilitate the spread of infection.
5. Sanitary condition of the villages.
6. Recommendations for the control of the disease.

In the present paper, it is proposed to deal with certain morphological observations on the cercariae found in the intermediate host, and with some facts relating to the bionomics of this snail which were not dealt with in the Government Report.

Major Connolly, to whom the various snails collected were sent and who kindly identified them, proposes to publish a complete account of all the snails found during the expedition and to include in it all previously known forms found in Sierra Leone.

DESCRIPTION OF THE CERCARIA OF *S. HAEMATOBIUM*

Examination. The snails were placed each in a separate small test-tube in clean water ; the cercariae which emerged were examined with a hand-lens of 20 magnification in order to study their movements. The fluid containing them was then pipetted off as required and mounted as a cover slip preparation, and the details of structure noted. It was found that cercariae which emerged just after clean water was added to the snails were more easily studied than those which emerged into water which had stood some time ; in the latter case the medium was not so translucent as in the former. The living preparation gave the most satisfactory results for the study of the suckers, the secretory glands and the excretory system, especially the flame cells. On the other hand, fixation with subsequent staining gave better results in studying certain features of the secretory gland cells and their ducts, the nervous system, the genital cells and cuticular spinulation. Specimens stained *intra vitam* were examined, the stain chiefly used being very dilute Leishman's stain.

Movement. In a tube of water the cercariae swim actively but intermittently ; as a rule, active progression is towards the surface of the water, the tail end leading. On cessation of swimming the furcal rami are usually held at right angles to the tail stem, and the animal begins to sink slowly. It was observed that if the rami were maintained at right angles (fig. 3, VI) the animal would quickly resume its upward movement towards the surface after a very short rest. If, on the other hand, the animal allowed the rami to curl inwards towards the tail stem in the shape shown in fig. 3, VII, it would fall rapidly in the water for a long distance. The downward movement was accelerated by curling the rami more tightly, until they appeared as two small knobs at the end of the tail stem.

Under a coverslip in a drop of water, the movements depend upon the amount of fluid ; if the fluid is deep the animal moves with great rapidity and passes out of the field. If the fluid is drained away slowly with blotting-paper, the animal attaches itself to the glass and progresses slowly by alternate protrusion of the cephalic end and drawing up after it of the body ; the tail, not being compressed by the slip, moves very actively at intervals ; it separates very easily

from the body in such conditions and was observed to retain the power of independent movement for a considerable period, up to ten minutes after separation. This independent movement was most frequently slow, affecting chiefly the base and rami, but sometimes a very active lashing movement affecting the whole tail was observed; in no case was translatory movement of the separated tail seen. The separated body, however, is capable of active progression and in this the ventral sucker is strongly protruded and adheres to the glass surface. Occasionally the animal appears to have difficulty in releasing its hold by the ventral sucker and the anterior end of the body is turned so as to release the ventral sucker by the help of the anterior protrusible organ. During the various movements, the body alters its shape constantly and remarkably and the anterior protrusible organ is pushed vigorously against any obstacle; the margins of the ventral sucker appear circular, oval, or linear in different views.

The appearance of the moving body under a coverslip changes so rapidly and the shape of the important organs alters so completely from one moment to another, that it frequently has little resemblance to the diagram which is reconstructed to represent the animal; the attached tail undergoes equally great variations in appearance, even when moving fairly slowly. It is only when the animal's movements are slowed down by removal of fluid from under the coverslip that the more important organs can be studied, namely, the anterior and ventral suckers and particularly the secretory glands.

Measurements. It is extremely doubtful if the measurements of the length or width of the body and the tail and rami of such cercariae can have any other than a crude comparative value. This statement is based on the fact that the animal in the living state is capable of such remarkable variations in the size of its body, tail-stem and furcal rami. The states of contraction and extension which are so obvious in the living animal are represented in fixed preparations. While a general contraction of the animal may be the result of applying to it certain fixatives, the degree to which the contraction affects different parts of the body will depend on the state of those parts of the body at the moment of application of the fixative. This is illustrated in Table I, in which are given the maximum and minimum sizes of parts of the cercariae.

TABLE I

The maximum and minimum sizes (in μ) of different parts of the cercaria in specimens, living, or fixed in different ways.

Measurement	Part of cercaria	Living		Fixed in Schaudinn's fluid		Fixed in 5 % formalin	
		maximum	minimum	maximum	minimum	maximum	minimum
Length ...	Body ...	242	105	196	121	189	114
	Tail stem ...	253	186	232	147	228	136
	Furcal rami	92	80	77	44	94	52
Width ...	Body ...	92	35	80	58	70	40
	Tail ...	46	23	42	33	47	24

It is evident that a comparison of measurements which show such variation in the extremes under each method can have only limited value. Even if we take averages of one method of fixation, *e.g.*, 5 % formalin, it is of little advantage, because the averages may be deceptively alike for any two groups, while the individuals vary enormously. Thus, taking two sets of fifteen examples fixed by this reagent from among those for which the extremes are given above, the result shown in Table II was obtained.

TABLE II.

Showing the average measurements (in μ) obtained from formalin fixed specimens of *S. haematobium*.

	Average length in μ	
	1st set of 15	2nd set of 15
Body	144	144
Tail stem	184	177
Furcal rami	63	67
	Average width in μ	
	1st set of 15	2nd set of 15
Body	57	59
Tail	32	34

The statements of Soparkar (1921) with reference to the cercaria of *S. spindalis* that

'the fully extended body often measures twice the contracted,'

of Sewell (1922) with reference to *Cercaria indica* XXX that it measures in body length extended 210μ and contracted 90μ, bear out the statement of Cort (1919) with regard to *S. japonicum* cercariae, that

'measurements of the cercaria of *S. japonicum* are very unsatisfactory data for comparison.'

Measurements of *S. mansoni* cercariae given by Iturbe and González differ so considerably from those given by Faust (1919) as to support the contention that measurements are of little service in differentiating the cercariae mentioned. The difficulty of attaining any standard agreement in regard to the measurement of such organs as the suckers is as great as that attending the measurement of the body, tail stem and of the furcal rami.

Cuticle. On the body, tail and on the furcal rami are present small backwardly directed spines; these are present also on the ventral sucker, but were not seen on the oral sucker. The spines are longer and more sparsely distributed on the base of the tail stem; subcuticular muscles both longitudinal and circular are present and are strongly developed in the region of the ventral sucker. There are no eyespots.

Anterior sucker. This is of elongated oval shape with a definite, thick, muscular wall, and is capable of a considerable range of movement, chiefly extension and contraction. In the substance of the sucker there is a homogeneous looking mass of a semi-fluid consistency, in the posterior part of which there are numerous small oval cells. There is no evidence of the presence of a nucleus, or duct, or secretion which would entitle this body to be termed a gland analogous to the head gland described by the Japanese observers in the case of *S. japonicum* cercariae. The mouth aperture is subterminal and is usually seen as a small circular opening from which a tube leads backwards into the body. At the anterior margin of the sucker, considerably anterior to the mouth opening, are situated the openings of the secretory gland ducts. Each duct opens at the tip of a minute refractile spine out of which the secretion can easily be seen passing in living specimens. From this point the

ducts can be traced backwards through the substance of the oral sucker, on its ventral aspect (fig. 2). There are five ducts on each side of the middle line. The ducts are markedly constricted where they pass through the wall of the oral sucker.

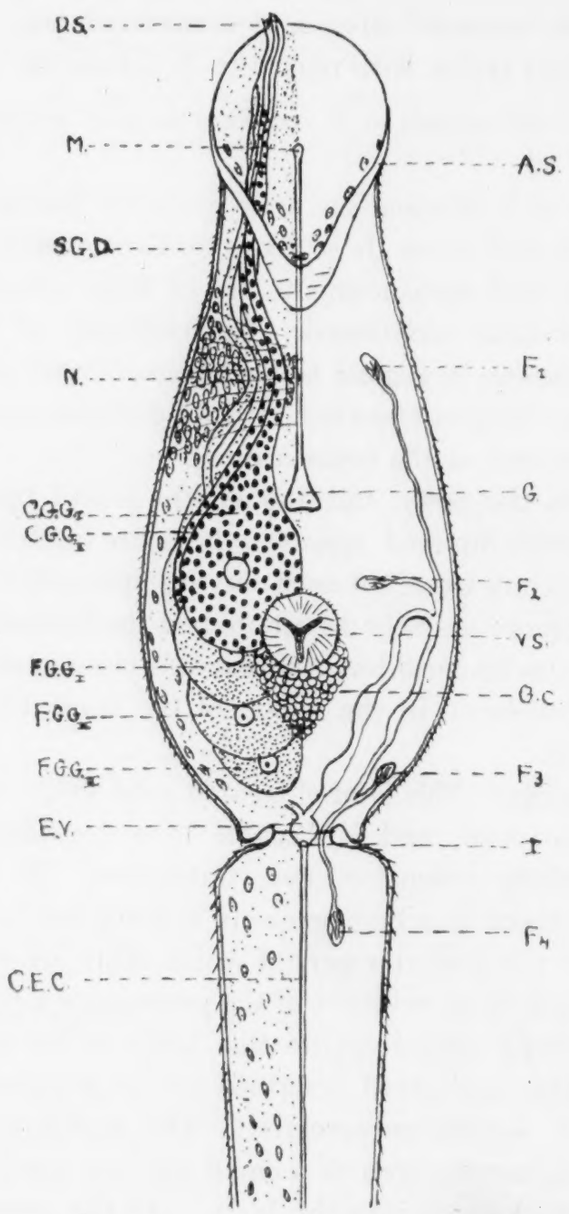

FIG. 1. Diagram of the Cercaria of *S. haematobium*. *D.S.*—Duct spines; *M.*—Mouth; *S.G.D.*—Secretory gland duct; *N.*—Nervous system; *C.G.G.*—Coarsely granular gland; *F.G.G.*—Finely granular gland; *E.V.*—Excretory vesicle; *C.E.C.*—Caudal excretory canal; *F.*—Flame cell; *A.S.*—Anterior sucker; *V.S.*—Ventral sucker.; *G.*—Gut; *G.C.*—Genital cells; *I.*—Island of excretory vesicle.

The Secretory Glands. The posterior two-thirds of the body are almost entirely occupied by pairs of unicellular glands. These glands in cercariae have received many names, *e.g.*, mucin, mucoid, poison, salivary, venom, lateral, proteolytic, cephalic, and secretory. Cort (1919) prefers to use the term ' cephalic,' since their ducts open at the anterior tip of the cercaria. As the term ' head gland ' is used by him for the gland in the anterior sucker of the cercaria of *S. japonicum*, this nomenclature is confusing. We use the term

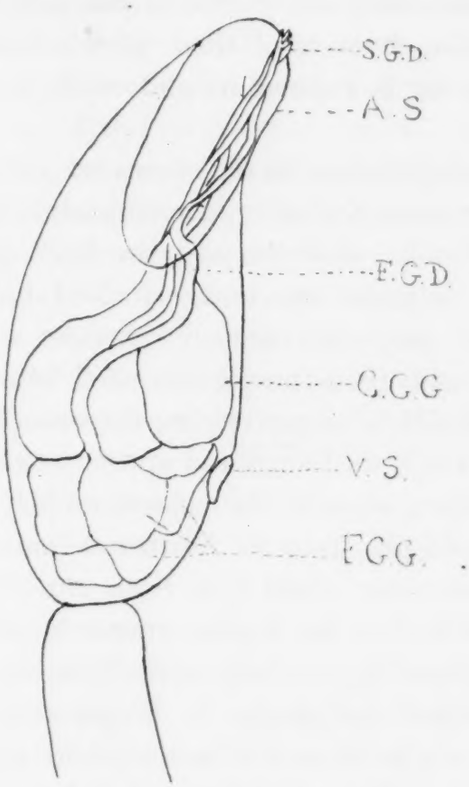

FIG. 2. Lateral view of cercaria of *S. haematobium*, drawn with the camera lucida, showing the course of the secretory gland ducts.

secretory glands for these body glands, in *S. japonicum*. The secretory glands number five on each side; they are unicellular, each with a single nucleus and definite nucleolus. The protoplasm of the cells contains granules; these are of large size in the anterior two pairs of gland cells, and small in the posterior three pairs of gland cells. The two pairs of anterior cells are large, generally flask-shaped, and lie about the same level, that is, just anterior to the ventral sucker. The three pairs of finely granular cells lie

posterior to them ; they are of smaller size and overlap each other somewhat.

The ducts from the secretory glands pass forwards ; widely separated at their origin from the individual cells, they become approximated in the region of the nerve cells and separate again before entering the anterior sucker. The ducts from the coarsely granular cells are also coarsely granular throughout their extent in the body and for a short distance in their course in the oral sucker ; they lie more ventrally and closer to the middle line than do the other ducts. The division of these glands into coarsely granular and finely granular is a constant and easily recognised fact in the living specimen.

The affinities of these cells for stains are variable, Stained with eosin for a short time, the anterior glands and their ducts often take on the stain diffusely, while the posterior finely granular cells remain unstained. In formalin specimens stained for prolonged periods with eosin, not only the coarsely granular cells, but the finely granular cells and their nuclei are well stained. In specimens stained with Ehrlich's haematoxylin, the anterior coarsely granular cells remain as a rule unstained and appear as a clear area in front of the ventral sucker, whereas the posterior finely granular cells with their ducts are deeply stained. With iron alum haematoxylin, the nuclei of the secretory gland cells both anterior and posterior are well defined and can be easily counted on careful focussing. The results obtained by staining were, however, not constant, even in fully developed cercariae. It is possible that the staining affinities of these glands vary to some extent with variation in their state of secretory activity. Faust and Meleney (1924) state that in the case of *S. japonicum* cercariae—in which the cephalic glands are all of one type—these glands are basophilic in reaction in very young cercariae. On reaching maturity the reaction changes from basophilic to oxyphilic, which condition prevails in the mature cercaria. Cort (1919) describing the same glands also in the cercaria of *S. japonicum*, states that they are stained a light blue with haematoxylin. It is interesting to note that the head gland of *S. japonicum* cercariae is described by Cort (1919) as taking ' the red stain erythrosin,' while Faust and Meleney (1924) state that it is slightly basophilic. Enough has been said to show that the acidophilic or

basophilic character of these cells is not a sufficiently definite nor stable one to justify its use as a distinguishing specific feature.

Alimentary Canal. This is simple in type and consists of a straight, narrow tube which passes from the mouth posteriorly and dorsally and ends in a small heart-shaped dilatation ; there is no pharyngeal bulb.

Excretory System. This commences in four flame cells on each side ; three of these are situated in the body as follows :—The anterior one half-way between the anterior extremity and the ventral sucker, the middle one on a level with the anterior margin of the ventral sucker, the posterior one about the level of the most posterior of the secretory glands. The fourth flame cell is situated in the tail near the base (fig. 1). From each flame cell a capillary leads off ; those from the anterior and middle body flame cells join ; those from the tail and posterior body flame cells also join. The two collecting tubes so formed unite at the level of the ventral sucker, curve ventrally and then pass towards the junction of the tail and body. Here they enter a globular excretory vesicle. From this a tube arises posteriorly which runs down the centre of the tail stem ; it divides into two branches just above the tail fork and each branch passes down one furcal ramus to open at its tip in a definite papilla. The flame cells in the body are seen most clearly from the dorsal aspect.

The excretory system corresponds closely with that of the cercaria of *S. japonicum* described by Cort and with that of *Cercaria indica* XXX described by Sewell. Cort (1919) found in addition ' two ciliated areas on each side near the ends of the sides of the bladder ' Sewell (1922) found two dilatations on each canal, each dilatation being provided with a ' flagellum.' The position of the flagellum in *cercaria indica* differs from that of the cilia in the cercaria of *S. japonicum*, as shown by the diagrams. We have not been able to satisfy ourselves of the presence of any flagellated or ciliated area in the collecting tubes of *S. haematobium*. In one specimen under observation from the lateral view, an appearance was noted which suggested the presence of such an area, but on rolling the specimen slightly it was found that the flickering movement seen was due to the movements of the flame cells of the opposite side showing through the body protoplasm.

Genital System. A triangular shaped mass of small cells just posterior to the ventral sucker represents the genital system.

Nervous System. There is in the region behind the anterior sucker a mass of cells which is wide at the lateral margins and tapers rapidly to cross the middle line as a narrow band ; in the substance of the mass, faint transverse lines are seen ; this appears, from analogy with other cercariae, to be the precursor of the nervous system of the adult.

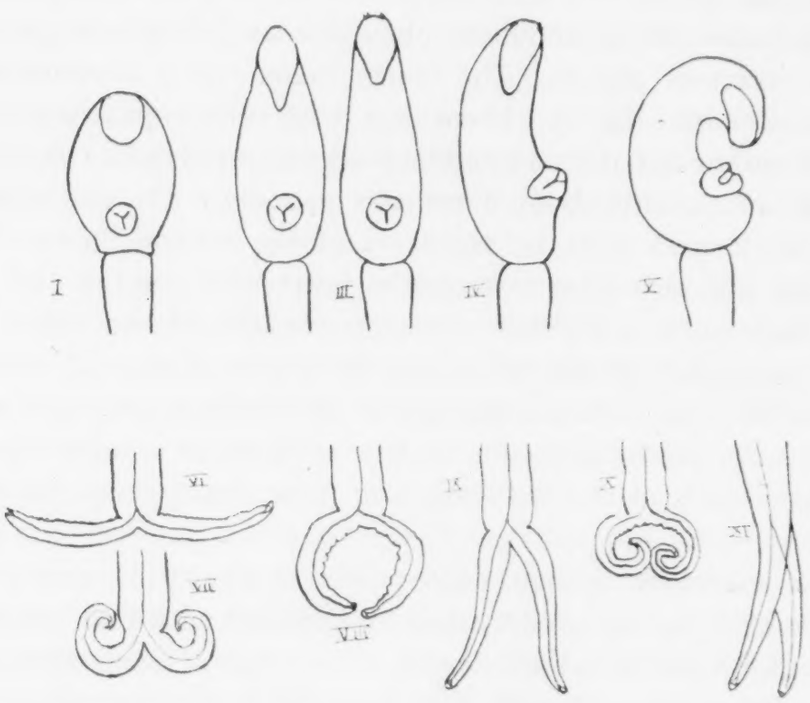

Fig. 3. Diagrams illustrating various positions assumed by the cercariae of *S. haematobium*.

The Ventral Sucker. This is small, being less than half the diameter of the anterior sucker. It is situated on the posterior fourth of the body. Its most characteristic feature is the remarkable degree to which it can be protruded. Seen from the side it has a cup-shaped appearance while from the ventral aspect it appears as a muscular ring with radiating lines and a central Y-shaped aperture. It is used by the animal for attaching itself to objects ; the anterior sucker is used freely as a penetrating organ.

THE DIFFERENTIAL CHARACTERS OF THE CERCARIAE OF THE THREE HUMAN SCHISTOSOMES

It has been shown that in the Schistosome cercaria from Sierra Leone here described, there are two sets of secretory glands comprising two with coarse granules and three with fine granules on each side of the body. The number, morphology, and position of the secretory glands in this cercaria are such as to distinguish it definitely from the cercaria which Faust describes as that of *S. haematobium*. On the other hand it agrees somewhat more closely, in the secretory gland arrangement, with his description of *S. mansoni*. He describes, however, six pairs of 'mucin glands' for the *S. mansoni* cercaria. In the number of glands the Sierra Leone cercaria agrees with the number for *S. japonicum* given by Cort; it differs in having the glands divided into coarsely and finely granular cells.

Faust (1919) gives the following table :—

TABLE III.

Diagnosis of Species of Human Schistosome Cercariae.

	S. haematobium	*S. mansoni*	*S. japonicum*
Size (in μ) :			
Body	240 × 100	140 × 60	100 to 210 × 66
Tail trunk ...	200 × 47	200 × 27	150 × 20
Furci	80 to 100 long	50 long	75 long
Oral sucker ...	60 in transection × 64 in length	30 to 34 in transection × 30 to 34 in length	33 in transection × 54 in length
Mucin glands ...	3 pairs with large nuclei and granular acidophilic cytoplasm.	2 pairs with large nuclei and granular acidophilic cytoplasm; 4 pairs with small nuclei and basophilic slime contents.	5 pairs with large nuclei and granular acidophilic cytoplasm.
Mucin ducts ...	Moderately thick.	Very thick.	Very thick.
Duct openings ...	At anterior end of oral sucker; capped by 3 pairs of hollow piercing spines.	At anterior end of oral sucker; capped by 6 pairs of hollow piercing spines.	At anterior end of oral sucker; capped by 5 pairs of hollow piercing spines.
Germ cells	Several large cells posterior to acetabulum	Many cells at posterior end of body	Clustered mass of cells just behind acetabulum
Parthenita	Sporocyst	Sporocyst	Sporocyst

Let us now consider these diagnostic points individually. Reasons have already been given for doubting the value of the *measurement* criterion.

The thickness of the *mucin ducts*, which are described by Faust as :—*haematobium*, moderately thick, *mansoni*, very thick, *japonicum*, very thick, can have no significance, because, as has been shown, the ducts of the secretory glands of the cercaria undergo great and definite variation in thickness at certain points during their course forwards to the cephalic extremity. The *duct openings* correspond in Faust's table to the number of glands allotted by him to each species ; they are each capped by a hollow piercing spine, and are situated at the anterior end of the oral sucker. The description of the position and number of the *Germ cells* given is not such as to afford much assistance in diagnosing the three species, nor is it of help in allocating the present cercaria. ' Several large cells posterior to acetabulum,' ' many cells at posterior end of body ' and ' clustered mass of cells just behind acetabulum ' may represent very similar numbers and arrangement of the Germ cells. The *Parthenita* in all cases is Sporocyst. It is seen therefore on analysis of this Table that the supposed differences which are set out so far are indeed trifling and are valueless for diagnosis. Only in one particular, namely, the number and character of the *mucin glands*, does there appear to be a point of some value—*haematobium* three pairs, *mansoni*, six pairs, and *japonicum* five pairs.

It is legitimate at this stage to examine the basis upon which this diagnostic table was compiled. The most complete description of the cercaria of *Schistosoma japonicum* then existing was that of Cort (1919). When Leiper (1915) differentiated by experimental means the two forms *S. haematobium* and *S. mansoni*, he did not give any such detailed account of the cercaria as has since been attempted, for he considered that animal experiment gave the only satisfactory differentiation.

Faust (1919) studied specimens of cercariae from Natal believed to be those of *S. haematobium* and gave a description which is too long for complete quotation.

' The oral sucker leads into a digestive tract without any evidence of a pharynx. An oesophagus runs backwards into *ceca which extend about three-fifths the distance caudad* (italics not in original). Paired groups of mucin glands empty their slimy contents at the outer margin of the oral sucker. Each duct opens thru' a hollow

piercing spine which caps the duct. Each group can be traced back to three mucin glands in the region of the acetabulum. These cells have loosely scattered granules in the cytoplasm and large nuclei. No other mucin glands have been found. Several germ cells have been found in the region of the body posterior to the acetabulum. The number is considerably in excess of the number of testes in the adult worm.'

This description, it will be observed, was made from dead material sent by Dr. Cawston; the material also contained cercariae which Faust described as those of *S. mansoni*.

There are certain facts of great relevance which may be brought into evidence with regard to the description of *S. mansoni* given by this author. He examined specimens of *S. mansoni* cercariae from Caracas and found that

'the mucin glands consist of only two pairs of cells of the granular type, but, in addition, four pairs of a non-granular type, somewhat smaller and surrounding the granular cells.'

This description is markedly at variance with that given by Iturbe who figures the glandular system of *S. mansoni* as having only three glands on each side. Faust (1920) states that

'this method of distinguishing between these species of larvae makes it possible to diagnose two species in material which Dr. F. G. Cawston has sent the writer from Natal, namely, cercariae of *Schistosoma haematobium* and those of *S. mansoni*. The latter species corresponds both by structural and micro-chemical tests to Iturbe's species from Venezuela.'

When we pursue this matter a little further, we find that Cawston (1920) is of the opinion that 'the statement must be taken with caution.' Later Cawston (1922) makes further reference to this subject and to Faust's diagnosis, as follows:

'In a specimen of *Physopsis africana* which I sent him from Natal, Faust has reported the presence of the cercariae of *S. haematobium* and *S. mansoni*, as well as *Cercaria octadena* which he regards as a developmental stage of *S. bovis*—it is difficult to understand how one individual snail from the Durban suburbs can have been exposed to infestation by the miracidia of all three Schistosomes.'

Faust (1921) met this criticism in so far as *S. mansoni* was concerned by pointing out again that the cercaria described by him for South Africa corresponds in all critical points with his description of the one from Venezuela which is known experimentally to be the larva of *S. mansoni*. Some explanation of this kind was clearly demanded as Cawston had drawn attention to the curious fact that although *S. mansoni* infection in human beings was not known to him to occur endemically in Natal, yet a single specimen of a snail harboured

the cercaria of *mansoni* as well as that of *haematobium* and of *bovis*. Further there was the additional cause of surprise to Cawston that the cercaria discovered in such interesting circumstances should be found in a type of snail host, *Physopsis africana*, which was never previously reported to contain the cercariae of *S. mansoni*. Faust would certainly appear to have been fortunate in obtaining in one snail specimen no less than three distinct species of schistosome cercariae and the fact that two of these were of great importance as being the early stages of Schistosomes affecting human beings gave his findings additional weight. The discovery by this means of the hitherto unsuspected endemic existence of *S. mansoni* in Natal was possibly of great epidemiological significance. Incidentally the revelation of a new type of snail intermediate host for *S. mansoni* was not less striking.

We find then that Faust's description of the cercaria of *S. haematobium*, is based on no definite evidence that he was in fact dealing with the cercaria of this species. It was not proved experimentally to be associated in any way with *S. haematobium*. The only evidence that it might probably be the cercaria of this adult worm was that it came from a likely snail from an endemic area. But the very fact that it came from a snail which was an intermediate host of *S. haematobium*, appeared to Cawston somewhat strong evidence against there also coming from this snail a cercaria which was the young stage of *S. mansoni*. In 1921 Faust, to meet the objections of Cawston, and in order to clinch the argument finally, made the following statement with regard to the same snail :—

> ' Furthermore, I have actually seen the lateral spined eggs of *S. mansoni* preserved in the liver gland of *Physopsis africana* which Dr. Cawston collected from Ottawa, Natal, and later sent me.'

Faust does not suggest any possible or probable explanation as to the means by which these eggs arrived in the position stated in the snail.

Thus detailed descriptions of cercariae which he presumed were those of *S. haematobium* and *S. mansoni* have been made by Faust ; in both cases from preserved material and without the advantage of studying living material. The great necessity of examining living specimens is emphasized by Cort, Sewell, Soparkar, and others who have added materially to our knowledge of the minute anatomy of cercariae.

THE IDENTITY OF THIS CERCARIA FROM SIERRA LEONE

We are faced by the fact that the description of the cercaria as given above differs from the description of the cercaria of *S. haematobium* given by Faust and reproduced in various text-books. It differs from his description not only in the number and character of the secretory glands but also in the anatomy of the alimentary canal. If this author's description is correct, then we are dealing here not with *haematobium* but with some other species. But the evidence provided by the epidemiology, by the experimental infection of snails, by the type of snail infected, and last of all by the results of the experimental infection of laboratory animals appears too convincing to be set aside, and we have little doubt that this cercaria is, in reality, that of *S. haematobium*.

The evidence which can be given to prove that it is the cercaria of *S. haematobium* is :

A. Incidence in Human Host.

Among the population examined no schistosome ova other than those with terminal spines were found. In a total of 180 faeces examinations these ova were found once ; among a total of 808 urine examinations they were found in 305 persons.

B. Experimental Infection of *Physopsis* c.f. *Globosa*, Morelet

Nine specimens of this snail from an uninfected area were kept in separate tubes and examined daily. Each day fresh water which had been boiled and then allowed to cool was used to replace that in which the snails were kept. The snails did not give out any cercariae ; controls which were dissected did not show any infection. The terminal spined ova from the urine of an infected case were then added to the tubes in which the snails were kept, on two successive days. Active miracidia were seen attaching themselves to the snails. The water thereafter was daily examined as before and then changed when found free of cercariae. On the fifteenth day after the second exposure, cercariae were found in the water in which one of the snails was. Two others also were found to be infected during the next examination. Dissection of the remainder of the snails did not reveal any others infected. The cercaria found in the case of each of these snails was identical with the cercaria described here

from naturally infected snails. This experiment is of course open to very obvious objection if it were the only evidence provided, but as purely corroborative evidence it has some value. It is of interest to note that the cercariae from one of these snails were used to infect a monkey and that from this animal the adult male form of *S. haematobium* was recovered.

C. Incidence in Snail Host.

Planorbis (*Hippentis*) species was found in only one village out of 30 examined, and of 318 specimens dissected none harboured this cercaria, although 151 were infected by cercariae of other kinds.

The snail here found associated with the presence of the disease in human beings was this *Physopsis* sp., nearly akin to *Pl. globosa*, Morelet. This was also the only snail found infected with the cercaria described. The infection rate in these snails with this cercaria was frequently high and was particularly so when the snails were taken in water latrines; in one case 42 per cent. of 50 snails taken in such a site were infected with this cercaria.

D. Experimental Infection of Laboratory Animals.

From guinea-pigs and monkeys which were submitted to scanty infection with this cercaria by feeding, adult males morphologically identical with *S. haematobium* were recovered after a period of three months.

Such is the evidence which makes it probable that this cercaria is that of *S. haematobium*.

Reverting now to the differential characters given in Table III it appears that the only one which seemed to be definite has broken down in so far as concerns the cercaria of *S. haematobium* and that we are left with very little upon which to base a diagnosis between the three species of cercariae which affect human beings. The gland arrangement in *mansoni* will doubtless receive further attention and should it be proved from careful study of living specimens that the cercaria of *S. mansoni* possesses five glands on each side, then we shall have reached again the stage at which, pending the production of further morphological facts or other criteria, we must diagnose the cercariae by the experimental method.

THE INTERMEDIATE HOST

Bionomics of *Physopsis*.

Some observations which appear worthy of recording were made on this snail. The snail was found only in certain situations; in water which lay or ran slowly on a muddy bottom, where weed or grass grew in the water, and under high or low shade; it appeared that these three factors—mud, weed, and shade—were essential to it in the water of the localities in which it was found (Pl. XIV, figs. 1 and 2). It was never found in a stream which had a clean sandy bottom, even though weed and overhead shade were present. Further, it was often absent in places which looked at first sight admirable sites for it, in water that is to say with shade, mud and weed; examination proved that the mud layer at the bottom of the water in these places was only a thin layer lying over sand. This snail likes mud, and the mud-living habit doubtless explains its distribution in the country traversed. In the hilly country, the stream bottoms were covered with sand, and there was no silting of mud. Consequently, this snail was not found at the villages situated round the base of the Loma mountains. Indeed, in the mountainous Koranko country, it was only found on the route traversed at two places. Sokurella and Benikorro, both in relatively low lying country. Its habitat is important from the point of view of prophylaxis against Schistosome infection. It may be mentioned that it was found in large numbers in a wet rice field at Jiama, which was practically marsh land; on the other hand it was never found in open, quickly flowing streams, in the same locality.

Infection of *Physopsis* with Cercariae of Human Schistosomes

Of 1557 specimens of *Physopsis* dissected, 306 had cercariae of some kind present in their tissues. Out of the 306 there were 184 infected with the cercariae of *S. haematobium* which was, as shown above, the only Schistosome affecting the population examined. In some cases where cercariae of *S. haematobium* were found, other cercariae were also present in the same snail. On the other hand, in snails from some localities, little or no infection of any kind was

found ; the variable rate of infection from different localities was a very striking phenomenon. Examples of this are given in Table IV in order of increasing infection.

TABLE IV.

Giving infection rates in *Physopsis*

Locality	Number of *Physopsis* dissected	Number infected with cercariae of *S. haematobium*	Percentage
Jiama (Nimmi Yemma)	497	12	2·4
Jiama (Nimmi Korro)	200	18	9·0
Bendu	160	18	11·2
Taiko	48	8	16·6
Kaiyima	373	112	30·0
Paya...	50	21	42·0

These high rates of infection in *Physopsis* may be compared with the figures which are given by Manson-Bahr and Fairley (1920) for *Bullinus* in Egypt. These authors discuss the reasons which may account for the fact that while it is difficult to find *Bullinus* infested in any numbers, the infestations of human beings with *S. haematobium* are very numerous. They point out that the highest infestation recorded by them in this snail for one month was 9 per cent., and that it more commonly was 1 or even less, per cent.

On considering the factors which result in the production of a high rate of infection in these snails, we found that the infection rate was directly proportional to the contamination of the water supply in a village by human excreta. The effect of even a small stream, if used for latrine purposes, in producing a high infection of *Physopsis* with cercariae of human Schistosomes is clearly seen on analysing the Jiama (Nimmi Yemma) figures. One section of the village goes to a spring for its drinking water. The path to this spring passes through a shallow, muddy pool into which comes the water from the men's latrine situated a few yards away (see fig. 4). From the spring a stream runs towards the village and turns sharply into a wet rice-field just before reaching the pool mentioned. The spring and the

upper part of the stream from it yielded no *Physopsis ;* the water ran on a bed of coarse sand. Lower down towards the village a layer of mud had formed on the sand and one *Physopsis* was found here. On following this stream into the rice-field where it became

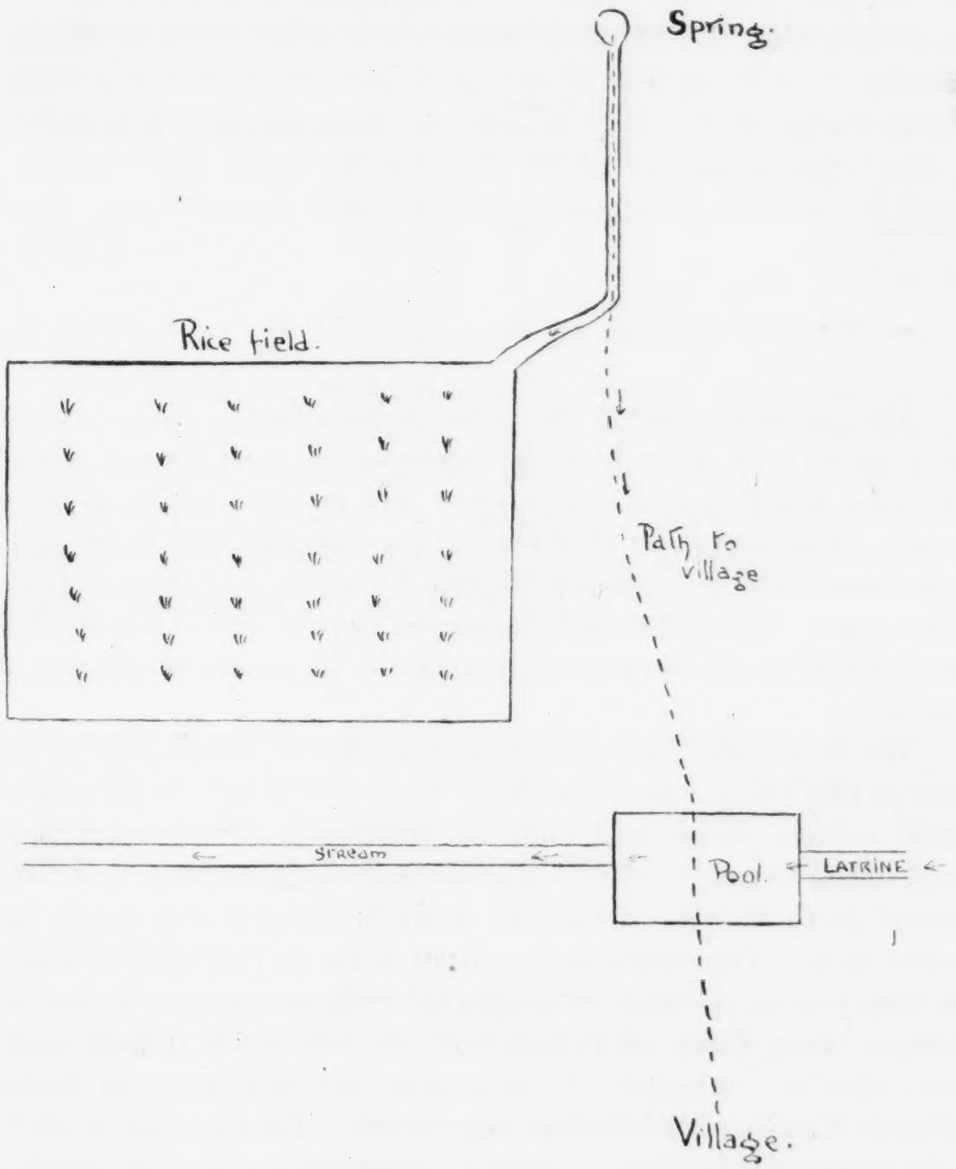

FIG. 4. Diagram of Spring, Rice-field, and Latrine pool at Jiama.

lost in marshy muddy ground, *Physopsis* was easily found, usually on weeds or on the underside of fallen leaves ; many were in the mud and came to the surface after the mud had been trampled down.

In the pool adjacent to the latrine (see Pl. XIV, fig. 1) *Physopsis*

was also found ; the pool water did not enter the rice-field but skirted alongside it for some distance. The result of dissection of snails from these different sources is illuminating, and is set out in Table V.

TABLE V.

Cercariae of *S. haematobium* found on dissection of *Physopsis* at Jiama (Nimmi Yemma).

Source	Number of *Physopsis* dissected	Number infected	Percentage
Rice field	430	1	0·2
Stream	1	0	0·0
Pool adjacent to latrine	66	11	16·6

The present position at this corner of the village is that every one in going to fetch water from the spring has to wade through a pool of water which is a veritable sewer and contains highly infected snails. This pool is an ideal one for the dissemination of cercariae since most children in passing through the water play about in it for some time. Situated in such favourable circumstances, the infected snail is able to do the greatest damage to the maximum number of people.

The facts noted here were confirmed almost immediately in the case of two villages in the vicinity, Paya and Taiko ; from each of these villages people had come for treatment. As a return they were asked to collect snails. The instructions given were that they should go to the place where the men's latrine was and search the water there. The results were :—from Paya, 50 *Physopsis* of which 21 that is 42·0 per cent. were infected with cercariae of *S. haematobium ;* from Taiko, 48 *Physopsis* of which 8 that is 16·6 per cent. were similarly infected. A messenger was next sent to Jiama (Nimmi Korro) with identical instructions. He returned 29.12.23 with nearly 300 *Physopsis* of which 200 dissected yielded 18 infected with human cercariae, that is 9 per cent. It is of interest to note that the pool at Jiama (Nimmi Korro) in which these snails were found, dries up in the dry season. A messenger sent there in April found only 6 *Physopsis* alive in the mud at this particular spot.

Physopsis then, in addition to being a mud snail here, is also

quite definitely a sewage snail, and that to a very marked degree. It was observed that those *Physopsis* from the water latrines were the largest and most active of all found, as well as being the most heavily infected with human Schistosome cercariae.

The fact that at Jiama the latrine snails were relatively few but heavily infected, while a few yards away the rice field snails were many and scantily infected, is one which may prove of considerable importance from the practical point of view. It is evident that a comparatively simple modification of the latrine arrangements of such villages should result in a great diminution of human Schistosomiasis.

Resistance of the *Physopsis* sp. to Drying

Experiments were made in order to ascertain to what extent the snails could survive alterations in their environment, especially by drying. The appearance of the snails after comparatively short periods of drying is deceptive ; the animal retracts very far into the shell and looks as if it were dead, but on immersion in water it expands slowly and resumes activity.

(1) Alternate Drying and Soaking

5 snails were placed dry in a glass dish in the shade. They were kept thus for a period of 20 hours and then placed in water. After 4 hours in water the whole process was repeated. All 5 snails survived 2 days of such treatment, and 3 survived over 3 days.

(2) Drying

2 snails glued on a card were exposed alternately to sun and shade for 48 hours. They were still alive, and moved actively after soaking for an hour-and-a-half in water.

3 snails were tied in dry muslin and placed in the shade. After 3 days they were put in water. 1 was alive. This one was subjected to alternate drying and soaking as in the first experiment. It survived until the 9th day.

5 snails were tied in dry muslin in shade. After 3 days 3 were alive ; dried and tested again on the 5th day, 3 were still alive ; on testing on the 7th day they were dead.

(3) Gradual Drying on Mud

18 snails were placed on wet mud in shade and the water was gradually drained away from the vessel. The snails remained on the surface of the mud adhering to it and retracted within their shells. On the 13th day all the snails were placed in water. After a long period 14 were alive and active. The first showed signs of life only after over an hour's immersion, and the last only after 10 hours immersion.

Depth in Soft Mud attained by the Snails

Many experiments were made to see whether exposure to sun on wet mud or on drying mud would cause the snails to bury themselves. In only one case did a snail go so deep as an inch-and-a-half from the surface, in the vast majority of cases they remained either on, or just under the surface. Direct sunlight was rapidly fatal to snails lying on dry mud.

These experiments prove that the resistance of this snail to various alterations in its environment is by no means negligible. It appears to be a much more resistant snail than *Isodora innesi* of which Archibald (1923) writes: 'if deprived of water for a period longer than six hours, it will surely die.'

The practical importance of the possession of such resistance by this *Physopsis* sp. is that merely cutting off or diverting the water for a few days in infected areas will not ensure the death of all or probably even of many of these snails.

It is of interest to note that in describing the present day conditions of the village of El Marg in Egypt, Faust and Meleney (1924) remark:

'as in the past the water supply was intermittent, being off for five days then on for a period. Snails of the species *Isodora truncata* were collected just north of the village. Leiper's (1915) recommendations have apparently not been carried out.'

One conclusion in the Report to the Government, arrived at as a result of the consideration of the local conditions, was that the proper and only promising methods of dealing with the problem of Schistosomiasis here was firstly by means of attacking the snail where it is known to be infected, and secondly, by so modifying, through education, local customs with regard to water pollution, as finally to prevent infection of snails. Any attempt here at an extensive and wholesale campaign of treatment by antimony or other drugs was deprecated as being, for definite reasons given, likely to defeat its own object and to be an expensive failure.

SUMMARY

(1) During an investigation into the prevalence of Schistosomiasis in certain districts of the Protectorate of Sierra Leone, infection due to *S. haematobium* was the only type of the disease found.

(2) *Physopsis* c.f. *globosa*, Morelet, was proved to be the intermediate host; the infection rate in the mollusc with cercariae of *S. haematobium* was often very high, *e.g.*, 42 per cent. in a water latrine.

(3) A description is given of the morphology of the cercaria of *S. haematobium*; it differs markedly from the description of this cercaria at present accepted.

(4) A critical analysis of the basis of the existing description is undertaken.

(5) Some facts relating to the bionomics of *Physopsis* c.f. *globosa* are mentioned; experiments showed that this snail was resistant to drying to an unexpected degree.

(6) Of snails placed on mud in the shade a large percentage survived for a fortnight when the water was drained away gradually.

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EXPLANATION OF PLATE XIV

Fig. 1. View of latrine pool at Jiama. (*Vide* fig. 4). Infected *Physopsis* found.

Fig. 2. View of washing and latrine pool at Kaiyema. Infected *Physopsis* found.

Fig. 3. View of men's bathing place on the River Kaiso. No snails found. Sandy bottom, no shade, quickly flowing.

FIG. 2

FIG. 1

FIG. 3

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EXPLANATION OF PLATE XIV

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FIG. 2

FIG. 1

FIG. 3

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OBSERVATIONS ON THE CLASSIFICATION OF CERTAIN SCHISTOSOME CERCARIAE

BY

B. BLACKLOCK, M.D.

AND

M. G. THOMPSON, M.B., B.Sc.

(*From The Sir Alfred Jones Research Laboratory, Freetown*)

(*Received for publication, 23 June,* 1924)

Since sending our description of the cercaria of *S. haematobium* to press, we have had the opportunity of seeing Khalil's paper on the cercaria of *S. mansoni* and Bettencourt and da Silva's paper on the cercaria of *S. haematobium* in Portugal; Khalil (1922) gives an account of the morphology of the cercaria of *S. mansoni* from *Planorbis boissyi* of Egypt. We find that his description of this cercaria corresponds almost in every detail with our description of the cercaria of *S. haematobium*. He describes, however, four oral glands with four ducts, but he qualifies this by the statement 'distinct outlines of separate glands cannot be made out' and that their duct openings are 'apparently unarmed with papillae.' He also describes two ciliated areas in the beginning and end of the main excretory duct. We referred in our description to our difficulty in making these structures out in the cercaria of *S. haematobium* and we also mentioned the variable accounts of these structures given by different authors. Bettencourt and da Silva (1922) found such areas in two fusiform dilatations in the tubes of the excretory system of the cercaria of *S. haematobium*, but noted that they were not easily observable in all specimens, and as a rule were not visible except when the cercaria was on the point of death. These ciliated areas are evidently so difficult to locate that they do not afford sufficiently reliable information for purposes of classification.

In Khalil's paper, describing the cercaria of *S. mansoni* from *P. boissyi*, the second figure in the text is labelled 'Camera lucida

drawing of *Schistosomum haematobium* cercaria.' This we conclude is a mistake for *S. mansoni*, for the following reasons :—

1. The title of the paper is ' Cercaria of *Schistosomum mansoni*.'
2. The material studied (p. 1) is from *Planorbis boissyi*.
3. The text is entirely a description of *S. mansoni* cercariae.
4. Khalil states (p. 5) : ' There is no reliable account of the anatomy of *Schistosomum haematobium* cercariae available.'
5. There is no figure of the general anatomy of the cercaria of *S. mansoni* except this, which is labelled *Schistosomum haematobium*.

Bettencourt and da Silva describe the cercaria of *S. haematobium* as possessing only three pairs of unicellular glands in the posterior region of the body ; the cells are of one type, having their protoplasm filled with coarse acidophilic granules. Three ducts lead forward from these and end in the openings. Our description of the cercaria of *S. haematobium* is at variance with that of Bettencourt and da Silva.

A study of this more recent work does not assist us in differentiating the cercaria of *S. haematobium* as described by us from that of *S. mansoni* as described by Khalil, by means of the morphology ; further, it does not assist us in distinguishing either of these from *Cercaria indica* XXX described by Sewell. By what means can we distinguish the three human Schistosome cercariae, *japonicum*, *haematobium*, and *mansoni* from each other and from *Cercaria indica* XXX ?

Morphology. If the secretory glands of *japonicum* are all of one type under all conditions and in all specimens, we have here a character to distinguish it from the other three. The most recent studies on the cercariae of *S. haematobium*, *S. mansoni*, and *C. indica* XXX disclose no constant morphological character nor staining reaction by which they can be distinguished one from the other.

CLASSIFICATION BY MEANS OF THE IMMEDIATE HOST

As our knowledge stands at present, classification of the above four Schistosome cercariae by means of mollusc hosts does not appear possible ; but a critical survey of observations on this point is interesting. Observers, however, are somewhat handicapped by the uncertainty and changeable nature of mollusc nomenclature. Annandale (1924) states that hitherto the cercariae of *S. japonicum*

have been found only in molluscs of the genus *Oncomelania*: 'Probably all the living species of the genus are potential carriers of *S. japonicum* but further information is needed on this head.' He states also that there is no evidence that *Blandfordia* is a disease carrier. Up to the present then, the cercaria of *S. japonicum* has only been found in the genus *Oncomelania*—but we do not know whether it will develop in *Planorbis* or *Bullinus*, nor yet whether *mansoni* or *haematobium* will develop in *Oncomelania*. Bettencourt and da Silva describe *haematobium* cercariae from *Planorbis* in Portugal. This observation appears to upset the possibility of classification by snail host. With regard to *S. haematobium* and *S. mansoni*, Leiper (1915) found that: 'In females reared from *Bullinus* the eggs are constantly terminal-spined, even in small young females. . . . In females reared from *Planorbis boissyi*, the eggs are constantly lateral-spined.' The work of Faust, Porter and the Portuguese observers, together with certain facts from India appear to show that classification by snail host is not now reliable; yet it seems remarkable that in Egypt where there are both forms of these human miracidia present and snails of both *Bullinus* and *Planorbis* genera, there has never been reported, so far as we are aware, any deviation from this selective habit. The geographical distribution of the infections does not suggest that the genus *Planorbis* can be generally utilized to propagate *haematobium*.

Before we can definitely state that classification by the snail host must be discarded, it appears to us essential that very complete experimental evidence must be brought forward which will associate clearly the cercariae from any supposed snail host with the adult worm. While the classification of the cercariae belonging to *S. haematobium* or *S. mansoni* appears to us impossible on the grounds of morphology or staining reactions, we are not in a position to state at present whether the snail host will finally help us in this matter.

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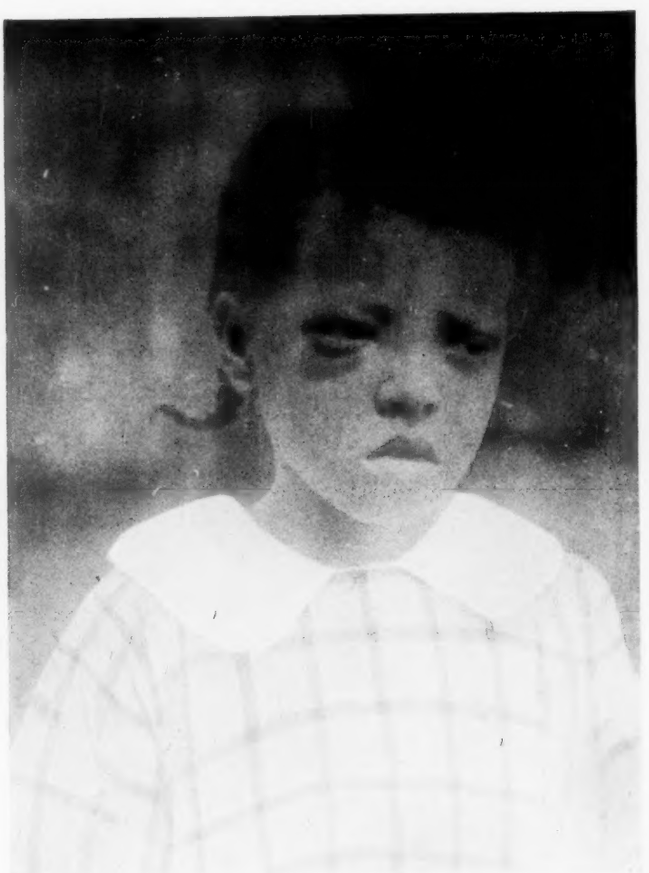

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MISCELLANEA

LARVA OF THE TUMBU-FLY *CORDYLOBIA ANTHROPOPHAGA* IN THE LOWER EYELID

PLATE XV

This case was shown to me by Dr. Wright of Freetown, under whose care it was. The larva, about one-third grown, had been removed some time before the photograph was taken and the swelling had largely subsided.

The Sir Alfred Lewis Jones Research Laboratory,
11.6.24.

B. BLACKLOCK, M.D.

THE HATCHING *IN VITRO* OF THE EGGS OF *OXYURIS EQUI*

It is well known that the embryonated eggs of *Oxyuris equi* occasionally hatch in saline solution, but the phenomenon is an inconstant one, and Schwartz (1923) who has recently studied it, concludes that it is purely accidental. The larvae which emerge are usually motionless or show only feeble or transient activity, and as pointed out by Schwartz they often are nipped by the too narrow opening in the egg shell and fail to emerge completely. It would seem that this phenomenon is capable of a purely mechanical explanation, for we have observed it in the case of eggs which had been kept dry at a temperature of 27° C. for over seven months, in which the embryos, although well preserved, showed no signs of life. Some experiments were, therefore, carried out to determine if more

satisfactory results could be obtained with media, approximating in some respects more closely to those which, if the eggs were ingested, would be met with in the alimentary canal.

The eggs used were collected from the margin of the anus of a horse and contained active embryos: at the time when the experiments were made they had been kept dry, at a temperature of 27° C., for from one to two weeks. It was found that immersion of such eggs in an acid solution of pepsin for twenty minutes did not cause them to hatch, but that if they were then transferred to either a solution of pancreatic juice or to a saline solution of approximately equal alkalinity, a large number of active larvae emerged. As the result of further experiments, of which that given in the table is an example, it was found that immersion of the eggs first in an acid solution (Hydrochloric acid, 0.1 to 0.2 per cent.) for half-an-hour or longer, and then in an alkaline solution (Caustic Potash or Sodium Carbonate from 0·1 to 0·5 per cent.) invariably caused large numbers of active larvae to hatch, whereas the acid solution alone and the alkaline solution alone failed to produce this result. On comparing the action of an acid pepsin solution with that of a corresponding solution of acid alone, little difference was observed, but in one or two experiments the number of eggs which hatched in the former was rather greater.

Experiment to test the action of acid followed by alkali on eggs of *Oxyuris equi*.

Time	Normal saline solution		Hydrochloric acid, 0·1 per cent.	
5·15 p.m.	Experiment started		Experiment started	
5.45 p.m.	No free larvae	Some of the eggs transferred to 0·5 per cent. sodium carbonate solution.	No free larvae.	Some of the eggs transferred to 0·5 per cent. sodium carbonate solution.
6.10 p.m.	No free larvae	No free larvae	No free larvae	No free larvae
6.50 p.m.	No free larvae	No free larvae	No free larvae	A few larvae have hatched, most of them actively motile
8.10 p.m.	One free larva; appears dead	One free larva; appears dead	No free larvae	Very many larvae have hatched

R. M. Gordon and J. W. S. Macfie.

The following parasites have been identified from animals which died at Sierra Leone.

Taenia taeniaeformis (Batsch, 1786) Wolffhügel, 1911.

Synonym : *T. crassicollis*, Rud., 1810.

Numerous specimens from domestic cats. The large hooks measured 385μ in length and the small hooks 225μ.

Cysticercus fasciolaris, Rud., 1808.

Two specimens from the liver of a rat.

Dipylidium caninum (Linn. 1758) Rail. 1892.

Four specimens from a civet cat and numerous specimens from dogs.

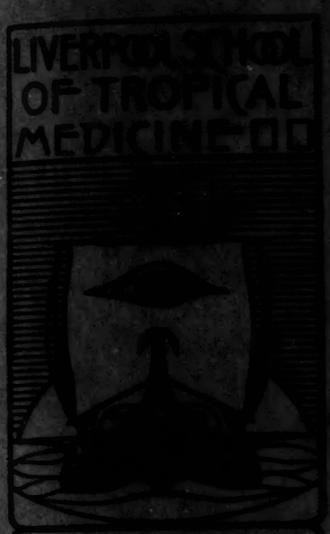
Hymenolepis diminuta (Rud., 1819) R. Blanchard, 1891.

Numerous specimens from rats.

T. SOUTHWELL.

UNIVERSITY OF LIVERPOOL

ctober 31st, 1924. XVIII. No. 3



ANNALS

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ON THE ASCARIDS OF THE DOG AND CAT

BY

E. L. TAYLOR, M.R.C.V.S.

From the Parasitological Laboratory of the Liverpool School of Tropical Medicine.

(*Received for publication* 9 *June*, 1924)

On looking through the collection of the *Ascarids* of the dog and cat at the Liverpool School of Tropical Medicine, four of the several specimen jars of worms from the cat were found to contain a species of *Toxascaris ;* of these, two contained *Toxascaris* sp. together with *Belascaris mystax,* while the remaining two contained *Toxascaris* sp. only. As no species of the genus *Toxascaris* seems to have been previously recorded* in the cat, the specimens were examined more closely and were found to present the following specific characters : Length of male 19 to 65 mm., length of female 22 to 80 mm. Cuticle finely striated, the striations being a distance of 4 to 9μ apart. The labial pulp shows two anterior lobules, detached from the main pulp by a well-marked cleft, and presenting a shallow depression at their extremities. The cervical alae are long and narrow, gradually decreasing in width posteriorly.

The caudal extremity of the female terminates in an acute point, and the vulva is situated about the junction of the anterior and middle thirds of the body. The eggs are 70 to 80μ in diameter, globular or subglobular and have a thick, smooth shell. The caudal extremity of the male presents a number of papillae of which there are six post anal on either side, two sub-dorsal, one lateral, small and difficult to see, and two sub-ventral, while just behind the anus on either side is a large double papilla ; these last three may be regarded as a continuation of the row of preanal papillae. Anterior to the anus on either side is a row of twenty-five or more preanal

* Since writing this paper my attention has been drawn to the fact that Baylis (1924), has recently recorded the presence of *Toxascaris leonina* in the cat.

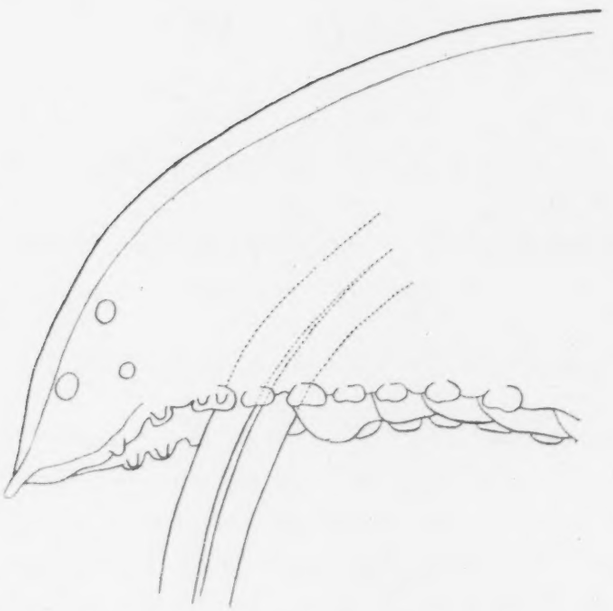

FIG. 1A. *Toxascaris*, sp. Cat. Caudal extremity, lateral view.

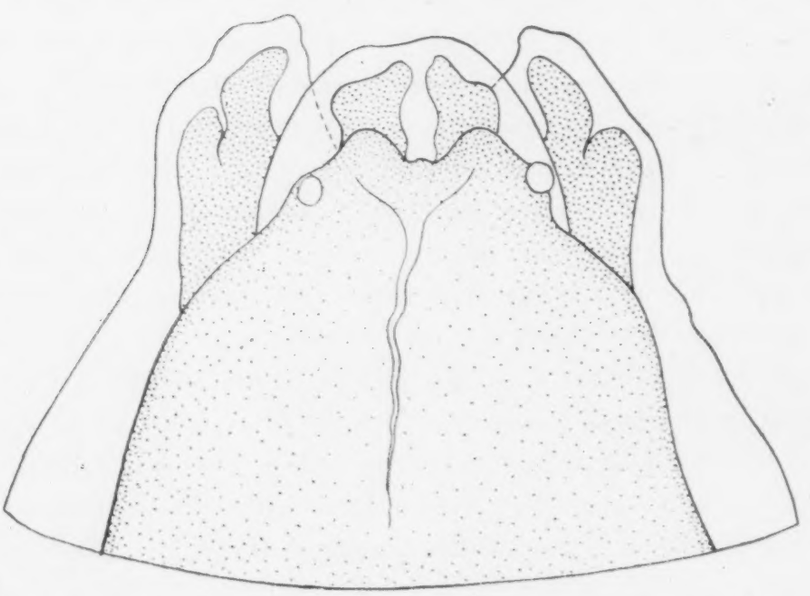

FIG. 1B. *Toxascaris limbata*. Head, dorsal view.

papillae, sub-ventrally placed; these increase in size posteriorly and towards the anus are large and can be much more readily distinguished than in the male *Belascaris mystax*.

The spicules of the male are 0·7 to 1·26 mm. in length and slightly unequal; they are not winged and are extended in about 25 per cent. of preserved specimens.

The lengths and other details of the worms belonging to the genus *Toxascaris*, collected from the four cats, are given in Table I.

Toxascaris limbata from the dog was now examined with a view to comparison, and measurements were made of the worms collected from four dogs. No difference in general morphology and microscopic appearance could be found. Details of measurements of these worms are given in Table II and it will be noticed in comparing these two tables that while the individuals vary greatly in size, there is no general difference between those from the two hosts.

Specimens of *Toxascaris leonina* from the lion were now examined, and, as with *Toxascaris limbata*, were found to differ in no particular from the species found in the cat. Details of measurements are given in Table III.

Railliet and Henry (1911) describe three species of *Toxascaris*, viz., *T. leonina*, *T. limbata* and *T. microptera*. Of the last species only two poor specimens were available and the description given is therefore very incomplete. *T. limbata* and *T. leonina* are described more fully and both descriptions seem to tally very well with the species of *Toxascaris* from the cat. Measurements of the two species made by Railliet and Henry are given in Table IV, and it will be seen that differences in size are very slight. The only other distinguishing features mentioned by these two observers are, firstly, that the caudal extremity of the female *T. limbata* terminates in a more acute point; and secondly, that the spicules in the male *T. leonina* are more frequently extended. These two relative characters seem to be of little value in distinguishing the two species. The comparative sharpness of the caudal extremity in *T. limbata* I have not been able to see, while the relative frequency with which the preserved male worm is found to have the spicules retained within the body or protruding from it seems to be a variable factor, since observations on a large number of specimens of *Belascaris mystax* and *Belascaris marginata* at my disposal, have shown the

spicules to be more frequently retained in the former than in the latter species, whereas Railliet and Henry found the reverse.

In my opinion, there is insufficient reason to warrant the division of the genus *Toxascaris* into the three species mentioned, and the

FIG. 2A. *Belascaris mystax*. Caudal extremity, lateral view.

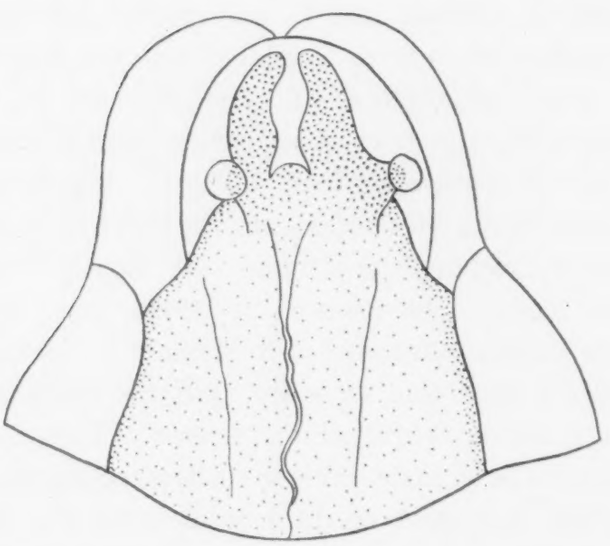

FIG. 2B. *Belascaris mystax*. Head, dorsal view.

Toxascarids of the lion, the dog and the cat are probably identical, and should be known as *Toxascaris leonina* (Linstow, 1902). To the three hosts mentioned, may be added the various hosts of

T. leonina given by Baylis and Daubney (1922), the tiger, leopard, ounce or snow leopard, fishing cat, leopard cat, hunting leopard and Indian fox.

Worms of the genus *Belascaris* from the dog and cat were found to fall into two distinct and definite species, viz., *Belascaris mystax*, confined to the cat, and *Belascaris marginata* confined to the dog. The most striking difference between these two species is seen in the spicules, which are relatively much larger in *B. mystax*, being about one twenty-fifth of the body length, while in *B. marginata* they are only one seventy-fifth of the body length. The difference between the cervical alae is also well marked ; in *B. mystax* they are broad, having their widest part near their posterior extremities, where they terminate abruptly ; while in *B. marginata* they are long and narrow and terminate gradually, resembling those of *Toxascaris leonina*. Particulars of measurements from numbers of these worms are given in Tables V and VI.

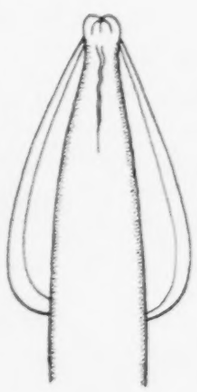

FIG. 3A.
Cervical aleae.—*Belascaris mystax.*

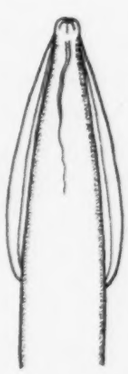

FIG. 3B.
Cervical aleae.—*Toxascaris limbata.*

FIG. 3C.
Cervical aleae.—*Belascaris marginata.*

I have not been able to satisfy myself as to the presence of the caudal alae mentioned by Railliet and Henry (1911) as very distinct in *B. mystax*. Several specimens when rolled under the microscope presented a dark longitudinal line on either side resembling the edge of a wing, turned upwards ; but on examining the same caudal extremities in section, the effect was seen to have been produced by the dark, somewhat opaque walls of the much flattened gut, which, seen on edge, through the cleared cuticle of the specimen might be mistaken for caudal alae.

TABLE I

Showing measurements of *Toxascaris* sp. from the cat.

Specimen bottle	Length of male in mms.	Length of female in mms.	Length of spicules in mms.	Distance between striations in μ	Size of eggs in μ
No. I	65	80	1·26	9	...
No. II... ...	40-50	47-75	0·80-0·95	7	60 × 81
No. III ...	19-25	22-23	0·70-0·87	4-5	...
No. IV ...	32	30	0·89	7-8	...

TABLE II

Showing measurements of *Toxascaris limbata* from the dog.

Specimen bottle	Length of male in mms.	Length of female in mms.	Length of spicules in mms.	Distance between striations in μ	Size of eggs in μ
No. I	30-35	40-60	0·95-1·04	5	60 × 75
No. II... ...	30-48	65-80	0·89-1·04	8·5	...
No. III ...	40-45	(*18)40-60	Old specimens could not be clearly seen	7·5	...
No. IV ...	55-70	75-100	1·11-1·27	7·4-14·6	74 × 86

*This is the measurement of one very small worm among a number of larger ones.

TABLE III

Showing measurements of *Toxascaris leonina* from the lion.

Specimen bottle	Length of male in mms.	Length of female in mms.	Length of spicules in mms.	Distance between striations in μ	Size of eggs in μ
No. I	37	50-68	1·12	8-9	60 × 85
No. II ...	28-52	29-63	1·00-1·50	4-10	66 × 75 to 70 × 84
No. III ...	37-43	39-70	1·05-1·20	4-11	66 × 75 to 68 × 84

TABLE IV

Showing the variation of length and other detailed measurements in the *Toxascaris* spp. from the cat, dog and lion, together with the measurements of *T. leonina* and *T. limbata* given by Railliet and Henry.

	Length of male in mms.	Length of female in mms.	Length of spicules in mms.	Distance between striations in μ	Size of eggs in μ
Toxascaris sp. from the cat	19-65	22-80	0·7-1·26	4-9	60 × 81
Toxascaris limbata from the dog	30-70	40-100	0·89-1·27	5-14·6	60-74 to 75-86
Toxascaris leonina from the lion	28-52	29-70	1·00-1·50	4-11	66 × 75 to 70 × 84
Toxascaris leonina [Railliet and Henry] ...	20-50	30-80	0·9-1·25	5-8	70-80
Toxascaris limbata [Railliet and Henry] ...	40-60	65-100	1·2-1·5	6-12	75-85

TABLE V

Showing measurements of *Belascaris marginata* from the dog.

Specimen bottle	Length of male in mms.	Length of female in mms.	Distance between striations in μ	Length of spicules in mms.
No. I	70-90	90-165	13-37	0·95 (extended)
No. II	70	67-170	12-24	1·05 (retained)
No. III	70	120-130	18-24	1·01 (extended)
No. IV	55	80-90	13·6-24	1·04 (extended)
No. V	50-75	50-120	9-27	0·74-1·3 (varying positions)

TABLE VI

Showing measurements of *Belascaris mystax* from the cat.

Specimen bottle	Length of male in mms.	Length of female in mms.	Distance between striations in μ	Length of spicules in mms.
No. I	60	85-108	24-37	1·98 (retained)
No. II	27-40	42-70	14·5-25	1·85-2·08 (varying positions)
No. III	53	80	18·5-28	2·08 (retained)
No. IV	...	63-85	18·5-29	...
No. V	42-70	90-105	10-27	1·63 (retained)

TABLE VII

Showing the variations in length and other details in *Belascaris marginata* and *Belascaris mystax*, together with those given by Railliet and Henry.

	Length of male in mms.	Length of female in mms.	Distance between striations in μ	Length of spicules in mms.
Belascaris marginata from the dog ...	50-90	50-170	9-37	0·74-1·05 (usually extended)
Belascaris mystax from the cat ...	27-70	42-108	10-37	1·63-2·08 (usually retained)
Belascaris marginata (Railliet & Henry) ...	50-100	90-180	16-22	0·75-0·95 (rarely extended)
Belascaris mystax (Railliet & Henry) ...	30-60	40-100	12-16	1·7-1·9 (generally extended)

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CRAW-CRAW IN SIERRA LEONE

BY

B. BLACKLOCK, M.D.

From the Sir Alfred Lewis Jones Research Laboratory, Freetown

(*Received for publication 23 June, 1924*)

PLATES XVI and XVII

Of all diseases seen in Sierra Leone natives, both in the Colony and Protectorate, none is more widely distributed and few are more productive of physical discomfort and chronic skin lesions than the disease called Craw-Craw in the Creole *patois* of the Liberated Africans. This disease has long been the subject of much speculation. Clarke (1848) who was Senior Assistant Surgeon to the Colony of Sierra Leone referred to it as Kru-Kru and it is here that we see the origin of the name. The description of the condition given by Clarke is :—

'KRU-KRU. This disease is characterised by an eruption which first appears in small pustules between the fingers, on the wrists, arms, hams, legs, and feet ; these pustules becoming confluent are ultimately spread over the body. It, however, sometimes occurs in separate patches on the neck, breast, back and hips, etc., when it is known among the Liberated Africans by the term Krooman's Kru-Kru. The itching and irritability are very great, which might be expected in a climate where so many debilitating causes exist. Yaws are often associated with this complaint and it is worthy of observation that leprotic disease not unfrequently follows a severe attack of Yaws. Kru-Kru occasionally occurs, in the persons of Europeans.'

Many years later O'Neill (1875) says of Craw-Craw :

'At first sight a well-marked case suggests the presence of extensive scabies in all its stages of development—the papule, the vesicle, and the pustule ; showing itself first in the clefts of the fingers, front of the wrists, and back of the elbows, seldom being found on the face, and always accompanied with intense itching. It should be stated that all the cases examined, about six in number, were in the persons of negroes, where the native hue of their complexion obscures the blush which, in the case of a "white man" would surround every eruption accompanied with irritation. The papules arise singly and at irregular intervals, increase to the size of a pin's head, feel firm to the touch, and, on account of the reason above stated, appear of the same colour as the surrounding integument. In some cases the papules arrange themselves in a crescentic form, like ringworm ; still it appears this is accidental, and that the separate and scattered distribution is the more common. In about two days' time the papule becomes converted into the vesicle, with very little increase in size, and in the course of a couple of days the pustule

is developed, rapidly enlarging, and uniting with those in its immediate neighbourhood. In the height of his suffering the patient tears the pustules, and their liberated and desiccated contents produce large and unsightly crusts. Night or day produces little alteration in the amount of itching, the cool of night tending, if anything, to lessen the irritation. The contagiousness of this disease is so well known that those affected are most studiously avoided. Three days' time is said to be the period at which the complaint shows itself after productive contact and it is popularly believed that, though a person affected should, in search of its riddance, proceed to the Cape of Good Hope or some other cool latitude, the disease which thus merely becomes latent will burst out with all its former vigour when the unfortunate patient returns to the warmth of the tropics. Sulphur, so beneficial in scabies, is here of doubtful efficacy, and the nostrums of the native "medical man" have frequently failed in bringing relief after six months' application.'

Kennan (1909) examined cases of Craw-Craw in Sierra Leone and in the Gold Coast Colony, and concluded that the disease, acute Craw-Craw, had an onset resembling that of an acute exanthem with a temperature at the earliest stage of 101-102° F. associated with definite constitutional symptoms—vertigo, headache, malaise. The rash which was vesiculo-papular in the earliest stages might be practically universal, but most commonly affected the exterior aspects of the arms and legs. Itching was not a prominent symptom in his cases and was commonly absent, especially in the earlier stages; when present later it usually disappeared before the rash had gone. He noted that all the cases seen were amongst natives and that they were mostly adult young men, not always of the poorest or dirtiest class. Kennan, as a result of his observations, concluded that the disease was contagious and that many cases are overlooked or diagnosed as scabies, etc.

Castellani and Chalmers (1919) say:

'Under the term Craw-Craw, African natives denote practically any pruriginous skin disease. Our African experience has taught us that most of the so-called Craw-Craw cases are cases of neglected scabies or of tinea corporis, or what Daniels and ourselves call cooly itch. *We apply the term Craw-Craw to a dermatosis met with in Africa, in Ceylon, and in various parts of the tropics, characterised by the presence of numerous hard, almost horny papules occasionally slightly exfoliating at the top, varying in size between a millet seed and a small pea. Some of the papules may be follicular. They are not of constant shape; some may be roundish and flattened, and others acuminate.*' [Italics not in original.]

Macleod (1920) says:

'Craw-Craw is a generic name which has been applied indiscriminately in West Africa both by white men and natives to various skin affections generally characterised by itching and pustulation. In 1875 O'Neill described under this heading a vesico-pustular affection which suggested scabies in its clinical characters and distribution. According to Bennett the uneducated natives of Calabar

employed the name for practically all skin diseases, while the more intelligent natives limited its application to three conditions, namely leprosy or bad Craw-Craw, Tinea circinata or Krooboy's Craw-Craw, and Craw-Craw proper, a papulo-vesicular disease which he believed to be pustular eczema. Under the same heading Plehn has described among the natives on the Cameroon coast a papular dermatitis which chiefly attacks the inside of the thighs. Emily has used the name in the French Congo for a chronic pustular disease which began as a reddish brown spot, was excoriated by scratching, and transformed into a superficial ulcer the floor of which gradually became covered with pale granulations secreting thick tenacious pus.

'The above are a few examples of the wide use of the term, but there are many others and except as an interesting native name it might well be abandoned.'

Manson's Tropical Diseases (1921) states that :

'the hard horny papules of craw-craw have to be differentiated from scabies, which is common in African natives.'

An opportunity was found in 1923-4 for observing large numbers of cases of Craw-Craw during an expedition through the Protectorate of Sierra Leone. The native names of several diseases are given below and it will be seen that Craw-Craw is distinguished in all these native tongues from leprosy, small-pox, and yaws. Each of these diseases is recognised clearly and the signs and symptoms are well known to the natives. The Creole word popularly used for small-pox is of some interest, ' Big Daddy,' *i.e.*, the old man, a term of great respect, denoting the legend by which the disease is associated, with the apparition of an aged man in the early epidemics.

TABLE I

Native Language	Name of Disease			
	Leprosy	Yaws	Small-pox	Scabies
Creole	Leprosy	Yaws	Big Daddy	Craw-craw
Temne	Arom	Katiri	Kabumbo	Tabool
Foulah	Barashi	Sareh	Badeh	Pohyeh
Mendi	Kpokpoli	Kewei	Bomboi	Nohoi
Limba	Teemo	Bongbo	Sambeh	Mootaki
Susu...		Suti	Senyak	Kasi

Those who were suffering from Craw-Craw in the villages were examined : the skin lesions produced for inspection by persons who stated that they were suffering from the disease were very varied and often severe, especially when the disease had been of long duration. In adults the most chronic and severe effects were seen in the gluteal and genital regions, in children carried on the mother's back the skin of the chest, abdomen and thighs were most affected ; in older children the finger clefts, hands and arms were the commonest sites. The only regions where the disease was not often seen were the head, palms and sole of the feet. No matter how old the infection, and notwithstanding the multiplicity of lesions of very diverse character which existed at the time of examination in the various parts of the body affected, there were factors common to all cases examined.

(1) The disease was characterised by itchiness.

(2) The sufferer, on being asked to point out the source of the trouble, invariably pointed to a minute papule or a vesicle which contained clear and rather viscid fluid.

(3) The small pustules frequently seen near the vesicles were considered no longer to contain the source of the disease, because scratched.

(4) The papules and vesicles which appeared to have an unbroken surface, were always found, with the hand-lens, to have a minute opening, usually central in position.

Especially in the gluteal region great thickening of the skin with the production of cicatrices, crusts and scales as a result of continual scratching and secondary infection was the rule. The armpit frequently presented a similar condition. But in small islands of healthy-looking skin the same small papules and vesicles would be indicated as the origin of the disease.

When the disease was fairly generalised over the body and of recent origin, the patient often had malaise and explained that he felt sick, that his stomach was out of order and that he had fever and headache. This condition was to some extent analogous to that described by Kennan as ' acute Craw-Craw ' except that in these cases, itching of the papules and vesicles was constantly complained about.

METHODS OF EXAMINATION USED

All that could be seen in the recent papule and vesicle was a small aperture surrounded by minute scales of dried epidermis. Dissecting out with needles was tried in the case of the fingers, the hand being placed on the stage of the dissecting microscope. In this way the parasite was discovered on several occasions, while on other occasions its eggs were found, and also the immature stages. In morphology the parasite found was not distinguishable from *Sarcoptes scabiei*.

The difficulty attending the discovery of the acarine of scabies in black skins has been referred to by Carlyle-Johnstone (1924).

In reviewing the Biology and Pathology of the natives of the Central Kavirondo District, Kenya Colony, he says :—

> 'Scabies, from which large numbers of the children suffer and which is not uncommon in adults, causes quite a lot of disability. As a result of scratching septic conditions often supervene. It is interesting to note that the causative organism of this disease has not yet been isolated. The cases were diagnosed as scabies on clinical grounds alone and they responded to ordinary sulphur treatment.'

CRAW-CRAW OR SCABIES IN SIERRA LEONE

The conclusion reached as the result of studying the history, and careful physical examination including the use of the hand-lens, was that the disease called Craw-Craw by the Sierra Leone Creole and the disease of which the various native names were translated as Craw-Craw is none other than scabies. The discovery of the parasite in many typical lesions of Craw-Craw cases confirmed this. It is usually assumed that the parasite of scabies is easily found. In my experience this is not so even in the white skin ; in the black skin the discovery of it is by no means easily made ; those who distinguish Craw-Craw from scabies state that the two conditions can be separated from each other since in Craw-Craw the mite is not found. It is not necessary to consider such conditions as that called Craw-Craw by Castellani and Chalmers. It seems rather like piracy to describe for Africa and Ceylon a new disease and use the name Craw-Craw for it in quite an arbitrary fashion. Craw-Craw, after all, is the Sierra Leone name of a disease which occurs in Sierra Leone and a very definite disease at that.

Its main characters are that it is a contagious disease which may affect persons of any age, and which takes the form of a definite eruption on the skin. It may be localised or generalised and any part of the body may be affected ; with the exceptions referred to above. It may have an acute onset and produce in its early stages definite constitutional symptoms depending upon the intensity of the infestation. The early lesion is a minute papule with an opening which is visible only by the use of a lens ; this is followed by a vesicle and this in turn by a pustule. The greyish burrow characteristic of scabies in the white skin is not observed in the black skin, nor is the faecal discolouration observed. This renders the diagnosis less obvious. If treated efficiently the disease stops at any stage so far described. But owing to non-recognition of the cause of the disease and to the appropriate treatment not being applied, the disease is allowed to assume a chronic form in which it is maintained by severe scratching ; this process assists not only in spreading the infection to healthy skin, but also produces from sepsis serious lesions in the affected area. Thus one sees in cases of some years standing affecting the gluteal and genital regions a very remarkable appearance. The skin is fissured and cracked, and covered with cicatrices and horny papules and ridges, an eczematous condition is frequently seen and ulcers of varying size. At this stage cases are difficult to treat and are often intractable because the skin condition is such that efficient treatment cannot well be tolerated.

Thus Craw-Craw here presents characters which are common to it and scabies as seen in England, but it presents additional features which result from prolonged and neglected infection, and which are seldom seen in England.

The recognition of the fact that Craw-Craw here is scabies is important, because as for scabies everywhere, early treatment of a somewhat drastic kind is essential for its eradication.

In the course of examination of patients for Craw-Craw, I found, as previous observers have done, cases in which the skin in the papules was infected with nematode larvae. These larvae are not the cause of Craw-Craw as they are frequently found in parts of the body which are not affected with Craw-Craw, and, furthermore, they are found in the normal looking skin of some persons who

are not suffering from Craw-Craw; they appear in these cases to give rise to no local or general symptoms by their presence.

SUMMARY

1. The disease called Craw-Craw in Sierra Leone is identical with scabies.

2. When the eruption is generalised and of recent origin, it sometimes corresponds to the condition described by Kennan as 'acute Craw-Craw.'

3. The parasite which gives rise to it is identical morphologically with *Sarcoptes scabiei*.

4. The parasite is frequently very difficult to discover in the black skin.

5. From long persistence in an untreated condition the disease often causes severe lesions in the skin.

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EXPLANATION OF PLATE XVI

FIG. 1. Craw-Craw affecting the thigh, scrotum and penis.

FIG. 2. Craw-Craw affecting the left armpit.

FIG. 3. Craw-Craw of buttocks, three months' duration.

FIG. 1

FIG. 2

FIG. 3

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EXPLANATION OF PLATE XVII

FIG. 1. Craw-Craw of buttock, one year's duration.

FIG. 2. Craw-Craw of wrist and hand, three years' duration.

FIG. 3. Craw-Craw of thighs, arms, and hands.

PLATE XVII

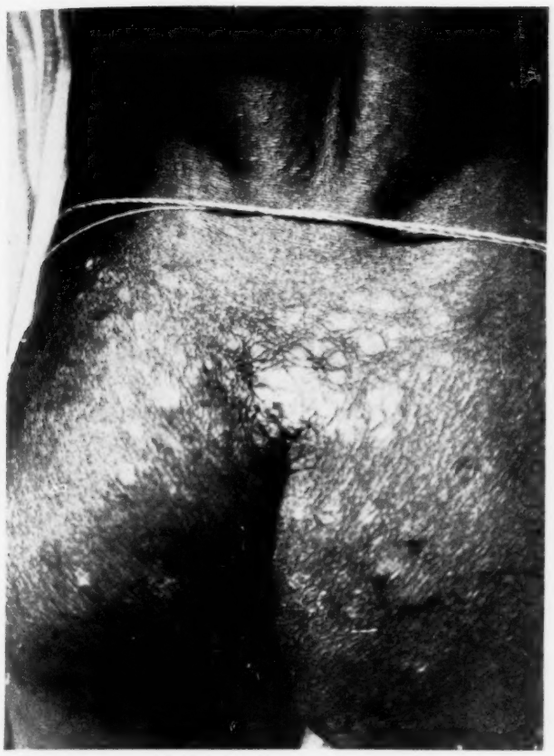

FIG. 1

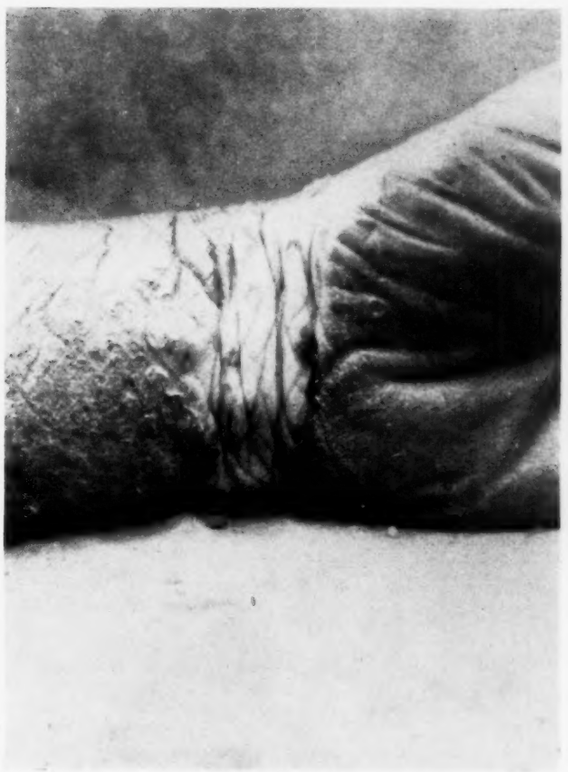

FIG. 2

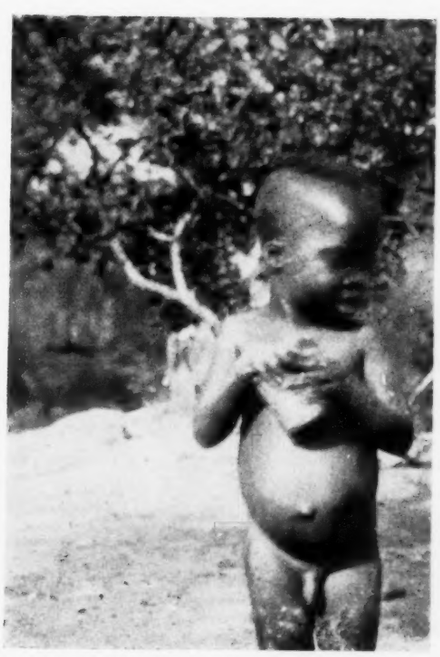

FIG. 3

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THE MOSQUITOS OF ACCRA

BY

A. INGRAM

AND

J. W. S. MACFIE

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The following list of the mosquitos which have been collected at Accra, Gold Coast, West Africa, is of some interest as showing the great abundance of these insects in some places in Africa.

All the sixty-eight species were found within the municipal boundary of Accra. This area, which is about three and a half miles long and two miles broad, is situated on the sea coast and surrounded on the land side by an arid plain, mostly bare, uncultivated, grass-land. It includes the town of Accra itself, the suburbs of Christiansborg and Adabraka and their immediate environs, two lagoons, but no forest land. The area would not, therefore, appear to be exceptionally favourable for the maintenance of a large number of different mosquitos.

The area has been rather thoroughly searched for mosquitos during the last few years, by Graham (1908), by Connal (1912), and by ourselves (1914 to 1923), and, moreover, is now-a-days very carefully watched by the Public Health Authorities, to one of whom, Dr. P. S. Selwyn-Clarke, we are indebted for the larvae of several species not previously recorded. Nevertheless, it is probable that other species still await detection, for up to the present additions have been made almost every year, and some parts of the area have not yet been thoroughly explored.

Finally, it should be clearly stated that, notwithstanding the large number of species known to occur in it, Accra is not a place where mosquitos are exceptionally numerous or troublesome; on the contrary, in the part where Europeans mostly reside they are comparatively rare, and the whole area must be regarded as usually relatively mosquito-free.

Mosquitos recorded as occurring within the municipal area of Accra :—

Aedes (*Aediomorphus*) *furcifer*, Edw.
Aedes (*Banksinella*) *fuscinervis*, Edw.
Aedes ,, *lineatopennis*, Lud.
Aedes (*Ochlerotatus*) *abnormalis*, Theo.
Aedes ,, *albocephalus*, Theo.
Aedes ,, *apicoannulatus*, Edw.
Aedes ,, *caliginosus*, Graham
Aedes ,, *domesticus*, Theo.
Aedes ,, *hirsutus*, Theo.
Aedes ,, *irritans*, Theo.
Aedes ,, *minutus*, Theo.
Aedes ,, *minutus* var. *biannulatus*, Theo.
Aedes ,, *nigricephalus*, Theo.
Aedes ,, *ochraceus*, Theo.
Aedes ,, *punctothoracis*, Theo.
Aedes ,, *tarsalis*, Newst.
Aedes ,, *wellmani*, Theo.
Aedes (*Stegomyia*) *apicoargenteus*, Theo.
Aedes ,, *argenteus*, Poiret (*Stegomyia fasciata*, F.)
Aedes ,, *luteocephalus*, Newst.
Aedes ,, *metallicus*, Edw.
Aedes ,, *simpsoni*, Theo.
Aedes ,, *unilineatus*, Theo.
Aediomyia africana, N-L.
Anopheles (*Anopheles*) *mauritianus*, Gpre.
Anopheles (*Myzomyia*) *costalis*, Lw.
Anopheles ,, *domicolus*, Edw.
Anopheles ,, *funestus*, Giles.
Anopheles ,, *marshalli*, Theo.
Anopheles ,, *pharoensis*, Theo.
Anopheles ,, *umbrosus*, Theo.
Culex annulioris, Theo.
Culex consimilis, Newst.
Culex decens, Theo.
Culex decens var. *invidiosus*, Theo.
Culex duttoni, Theo.
Culex fatigans, Wied.
Culex grahami, Theo.
Culex guiarti, Blanch.
Culex horridus, Edw. (*Protomelanoconion fusca*, Theo.)
Culex (*Micraedes*) *inconspicuosus*, Theo.
Culex insignis, Cart.
Culex (*Culiciomyia*) *macfiei*, Edw.
Culex ,, *nebulosus*, Theo.
Culex ornatothoracis, Theo.
Culex perfidiosus, Edw.
Culex perfuscus, Edw.
Culex quasigelidus, Theo.
Culex rima, Theo.
Culex thalassius, Theo.
Culex thalassius var. *fuscus*, Theo.
Culex tritaeniorhynchus, Giles.
Eretmopodites quinquevittatus, Theo.
Ficalbia (*Etorleptiomyia*) *mediolineata*, Theo.
Lutzia tigripes, Gpre.
Lutzia tigripes var. *fusca*, Theo.
Mimomyia hispida, Theo.
Mimomyia plumosa, Theo.
Mimomyia splendens, Theo.
Mucidus mucidus, Karsch.
Mucidus scatophagoides, Theo.
Taeniorhynchus (*Mansonioides*) *africanus*, Theo.
Taeniorhynchus (*Mansonioides*) *uniformis*, Theo.
Uranotaenia balfouri, Theo.
Uranotaenia connali, Edw.
Uranotaenia mashonaensis, Theo.
Uranotaenia mayeri, Edw.
Chaoborus ceratopogones, Theo.

NOTE ON A POSSIBLE INTERMEDIATE HOST OF *SCHISTOSOMA HAEMATOBIUM* IN THE GOLD COAST

BY

A. INGRAM

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Attempts have been made on several occasions during the past eighteen months to determine the molluscan host or hosts of *Schistosoma haematobium* in the Gold Coast by exposing Bullinus-like snails, collected in pools and water-holes used as bathing places in the neighbourhood of Accra, to the attacks of miracidia hatched from ova obtained from the urine of cases of Bilharziasis in the colonial hospital. Three species of such snails are usually found in these bathing pools and they are, according to the identifications very kindly supplied by Colonel M. Conolly : *Isidora forskali*, Ehrn, *Physa waterloti*, Germain, and *Physopsis globosa*, Morelet. *I. forskali* is of much rarer occurrence than either of the others.

Dissections of specimens of these snails made as soon as they were brought to the laboratory, appeared to show that neither *I. forskali* nor *Physa waterloti* was naturally infected with furcocercous cercariae and no furcocercous cercariae were found in the livers of these species after they had been exposed to miracidia of *Schistosoma haematobium* and had been kept alive for a month to five weeks. On the other hand, dissection of newly-arrived specimens of *Physopsis globosa* showed an infection with furcocercous cercariae in 1·2 per cent. (2 in 165 examined), and 14 of 25 survivors of a lot of 76 of this species, exposed to miracidia of *S. haematobium* on the 9th July, were found to be infected with furcocercous cercariae when dissected on various dates between the 23rd August and the 16th September, 1923. It has, nevertheless, to be admitted that all attempts to infect rats with these cercariae proved unsuccessful ; *e.g.* five *M. rattus* were placed in narrow glass cylinders and were kept for two hours semi-submerged in water to which had been added teased portions of livers of *Physopsis globosa* infected with furco-

cercous cercariae ; but although all the rats survived for more than six weeks after the experiment before dying or being killed, in no case were adult Schistosomata found in the liver or mesenteric veins. The dissection of three *M. decumanus* and of one *M. rattus* six to nine weeks after a similar exposure in water containing free-swimming cercariae, proved equally fruitless as regards the finding of Schistosomata in the livers or mesenteric veins, or the finding of ova in the bladders.

It may be noted that *Physopsis globosa*, Morelet, is the species of snail which Dye (quoted by Christopherson in a letter *Brit. Med. Jl.* of September 8, 1923, p. 437) in Nyasaland, observed to be attractive to the miracidia of *S. haematobium* and to be penetrated by them. It is, therefore, not unlikely that this species of snail can function as an intermediate host of *S. haematobium* in the Gold Coast.

THE CROONIAN LECTURES ON LEPROSY RESEARCHES

BY

LT.-COLONEL SIR LEONARD ROGERS, C.I.E., M.D., F.R.C.P., F.R.C.S., F.R.S., I.M.S.

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Lecture I. THE EPIDEMIOLOGY OF LEPROSY

When I had the great honour of being appointed to deliver the Croonian Lectures, I selected the subject of leprosy because I had spent my last few years in India in research on its treatment, and have since devoted several years to a study of its literature, while I believe it has not been discoursed on before this College since the late Dr. Robert Liveing delivered the Gulstonian Lectures in 1873, just when the epoch-making discovery of the lepra bacillus by Hansen was revolutionising our outlook on its etiology. Recent advances in treatment have at last furnished us with methods of dealing with the disease far more effectively than ever before, making the present time opportune for reviewing the whole subject. I propose in the first two lectures to deal with the epidemiology, mode of spread and prophylaxis of leprosy, and in the third to consider the improved methods of treatment.

THE SPREAD OF LEPROSY OVER THE WORLD

Munro, in his learned articles in the *Edinburgh Medical Journal* of 1877-79, refers to an Egyptian record of 1350 B.C. of leprosy among negro slaves from the Sudan and Dafur, which is interesting in view of the present high leprosy rates in Central Africa, while very early records of India and China are believed to refer to leprosy. The first reliable records of its spread relate to the invasion of Europe through Greece about 350 B.C., probably by the armies of Darius, while those of Pompey carried it to Rome in 62 B.C. Galen mentions it in Germany in 180 B.C., and it spread all over Western Europe by the ninth century; both Sir James Y. Simpson and Sir George Newman

have recorded much interesting information regarding early leper hospitals in the British Isles, while Bergen had a leper hospital in 1266 and still has one to the present day.

Leprosy was, therefore, widely prevalent in Europe before the Crusades, but greatly increased from the eleventh to the thirteenth century, only to show a remarkable decline in the latter part of the fourteenth century. This, I believe, was partly owing to the Black Death of 1349, which is said to have carried off nearly half the population of Europe, for recent records show reductions of leprosy in Iceland and India due to epidemic disease and famines falling most heavily on outcast lepers; but also due, I feel sure, to the drastic segregation measures enforced in Western Europe during the Middle Ages; for, as Munro and others have pointed out, the disease only declined rapidly and eventually died out in just those countries where segregation measures were carried out, and lingers to this day in countries which neglected them, such as Scandinavia, the Iberian Peninsula, Russia, Turkey, and Greece.

While the disease was declining in Europe it was carried to the Western Hemisphere by the Spanish and Portuguese invaders, and later by the extensive negro slave trade from the very parts of Central Africa which still show the highest leprosy rates, and more recently still by Indian and Chinese immigrants. All the evidence goes to show that the aboriginal Indians were free from the disease, and those having little or no intercourse with leprous races from the old world still remain free to this day. As late as the middle of last century serious outbreaks of leprosy took place in several of the Pacific Islands, Hawaii having been infected by a Chinaman in 1848, according to Hildebrand, who saw the first indigenous cases five years later. The outbreak in New Caledonia was certainly due to a Chinese leper, who died about 1865 after living two years with a tribe, one of whom developed leprosy only a year later, and the disease spread so alarmingly that, according to Ortholon, within ten years from one-fourth to one-half the people in some places were attacked, while by 1910 no less than 90 per mille of the 8,000 convicts had become lepers. The epidemic soon spread to the neighbouring Loyalty Islands, the first case again being in a Chinaman affected in 1878, and by 1909 Nicholas found 35 per mille of the population to be lepers, although many cases were hidden. The

Marquesas Isles were next invaded, and by 1909 the leprosy rate had reached the appalling figure of 66·7 per mille (Buisson), or two hundred times that of India. Professor Jeanselme stated in 1903 that the Chinese had also 'carried leprosy to Indo-China, Siam, the Straits Settlements, Java, Sumatra, Borneo, the Philippines and other East Indian Islands, in several of which countries Chinese still form by far the majority of the infected,' Sir James Cantlie as early as 1897 having pointed out the rôle of the Chinese in spreading leprosy in these areas.

It is therefore clear that leprosy still retains its powers of spreading, and I fear there is good reason to believe that, with the opening up of communications and trade routes, the disease is now extending in tropical Africa, so that the problem remains a serious one for the British Empire. Thus the whole history of the spread of leprosy over the world is that of an insidious and slowly communicable disease carried by armies or the emigration of infected races to previously immune countries, leaving no doubt regarding the infectivity of the disease in some way or other, and of the necessity of safeguarding against its further extension.

THE WORLD DISTRIBUTION OF LEPROSY AND CLIMATIC CONDITIONS

The close relationship between hot, humid climates and high leprosy rates, which I demonstrated last year by maps illustrating the leprosy rates per mille throughout the world and in India, respectively, is the most important epidemiological feature of the disease, as the following brief summary will show. Every area with the very high rates of 5 per mille and upwards (that is, fifteen or more times the present rate in India, where leprosy cases can be seen daily in the streets of every town) is situated within the tropics, having high annual rainfall, usually of sixty inches or more, *e.g.*, a large part of tropical Central Africa, with such extreme rates as 20 to 60 per mille on the Ivory Coast, the Cameroons and part of Eastern Belgian Congo, these constituting the most severely affected large area in the world ; while high rates are also met with in the hot damp countries of French and Dutch Guiana in South America, and in the Oceanic Islands, already mentioned as epidemically infected last century, and a few other areas.

In contrast with those high rates we have the remarkable fact that the only countries in the tropical zone, which have little or no leprosy, are the extremely dry areas of the Western Sahara, the French province of Mauretania, German South-west Africa, and the western coast of South America, all with less than ten inches of rain annually.

In the Northern Temperate Zone we also find the high leprosy rates of between 1 and 5 per mille in Norway and Iceland before active segregation measures were enforced, Japan and Korea, in all of which the rainfall is between 30 and 60 inches a year, against the usual rate for this zone of 10 to 30 inches.

Another important factor, however, comes into play in much of this zone, namely, the high degree of sanitary advance in Europe and North America greatly limiting the danger from returned emigrants infected with leprosy, with the result that the disease is no longer endemic in such countries.

The relationship between high humidity and relatively high leprosy rates and *vice versa* is illustrated in a most striking manner by my map of Indian leprosy distribution, rainfall and humidity data, the highest rates being met with in wet Burma, Assam, Bengal outside the deltaic region, Bihar, Orissa and the eastern Central Provinces, and the west coast of Bombay and Madras, which all have high monsoon rainfall, the leprosy rates in the Ganges valley actually decreasing with those of both the rainfall and the humidity in each division from east to west with the decreasing strength of the monsoon, while the humid Himalayan Kumaon Hills have ten times the leprosy rate of the hot, dry plains at their feet. On the contrary, the lowest leprosy rates are all met with in the dry North-western Punjab, North-west Frontier Province, Baluchistan and Sind, and in the dry central portion of the peninsula. The only exception is a somewhat high rate in comparatively dry Berar and the Deccan, probably due to infection by centuries of close trading with the people of the same race and language as those of the damp leprosy-infected western coast. Many other striking illustrations could be mentioned, such as higher leprosy rates in the rainy halves of Madagascar and Sumatra. I shall return later to the probable explanation of these facts.

INVERSE RATIO BETWEEN LEPROSY PREVALENCE AND TUBERCULISATION

The remarkable relationship I found between damp heat and leprosy prevalence led me to collect data regarding tuberculosis in the tropics, and to study the writings on the subject of Colonel G. E. Bushnell and Professor Lyle Cummins. These showed that climate itself had much less influence on this disease than the degree of tuberculisation of the population in any country, as illustrated by a high percentage of positive von Pirquet reactions indicating the numbers infected with mild or latent tubercle in childhood, protecting them to a large extent from the more acute forms of the disease in later life. It is now well established that native races in Central Africa, Oceana and America, among whom some of the highest leprosy rates are found, have very low percentages of positive tuberculin reactions, and are liable to suffer from decimating epidemics of very acute generalised tuberculosis on becoming infected from chronic cases of phthisis in Europeans. Thanks to Professor Calmette, von Pirquet reactions have been worked out in most of the French tropical possessions, and on studying these and other data I found that a low degree of tuberculisation of the people occurred in just those areas where exceptionally high leprosy rates per mille have been recorded, as shown in Table I ; intermediate rates are seen in such long tubercular infected countries as India and China, while the highest percentages of von Pirquet reactions are met with in Western Europe, from the greater part of which leprosy died out as an endemic disease over a century ago, in a manner which is unknown in any other part of the world.

In this connection it may be recalled that tuberculin gives reactions in leprosy, that such an acute observer as the late Sir Jonathan Hutchinson suspected that the tubercle bacillus may be modified by some element in badly cured fish to assume the power of producing leprosy, while such an eminent pathologist as the late Professor Sheridan in 1891 reported a case of leprosy with lung lesions in which he suggested the possibility ' that leprous lesions are the result of a *modified form of tuberculosis which tends to return to the normal type in the lungs.*' The italics are in the original. Although I do not for a moment endorse these views, they at least emphasise the close relationship of the two diseases, and suggest

as the most likely explanation of the interesting data in the table the possibility that infection by mild tubercle of a large proportion of a population may render it less susceptible to the infection of leprosy, this being one factor in the extinction of leprosy as an endemic disease in Western Europe, in addition to improved hygiene and other influences.

TABLE I

Leprosy Rates per Mille and von Pirquet Reaction Percentages.

Country	Leprosy per Mille	Von Pirquet Percentages
France (Lille)	0·0	87·7
Austria	0·0	95·0
United States	Extremely low	94·9
Indo-China	0·67	48·0
Martinique	1·16	57·0
India	0·32	Fairly high
French Guinea	5·0	12·0
Senegambia	10·0	15·2
Tanganyika	7·0	21·0
Cameroons	20·3	4·4
Ivory Coast	60·7	8·4

However this may be, the reverse is certainly not the case, as it is well known that the most frequent fatal complication of advanced leprosy is pulmonary tuberculosis, and one which greatly complicates treatment, for doses of chaulmoogra oil derivatives which are of value in leprosy are actively harmful to the tubercular complication, although sodium morrhuate in small doses may sometimes be used with advantage in such cases.

NUMBER OF LEPERS IN THE WORLD AND IN THE BRITISH EMPIRE

Some ten years ago Dr. Victor G. Heiser estimated the world's lepers at 2,000,000, since when further data have come to light, especially regarding the great prevalence in Central Africa, and a close study has convinced me that 3,000,000 would be a more correct figure, including the following recorded data :—

In EUROPE 7,000, with about 1,000 each in South Russia, the Baltic Provinces and Crete, and about 500 each in Turkey, Roumania, Spain, and Portugal.

In ASIA 1,250,000, including 500,000 to 1,000,000 in China with its dense population, 102,000 in both Japan and India, 15,000 in both Indo-China and Siam, 5,000 in both the Philippines and in the East Indies, and smaller numbers in Malaya, Ceylon and Palestine, etc.

In AFRICA 500,000, nearly all in tropical Central Africa, although the estimate is only a very approximate one, the data being nearly all based on the percentages found in the examination of a few thousand persons in different areas. Other recorded data are Egypt 6,500, Madagascar 4,000, and South Africa 2,500.

In the WESTERN HEMISPHERE 30,000, including Brazil 15,000, Guiana 3,000, Cuba 1,500, the British West Indies 1,000, Colombia 6,500, Venezuela 750, etc., while Oceana has about 5,000.

All the above figures relate only to typical advanced cases, while considerable infected areas remain for which no data are available, and it is safe to say that there is at least one early unrecognised case for every recorded typical one, Muir's Indian experience leading him to think that even this is an underestimate. Yet the recorded figures total 1,792,000, so that, including early unrecognised cases, 3,000,000 is far from being an improbable number for the whole world, constituting leprosy a problem of unsurpassed difficulty and importance when the terrible nature of the disease is borne in mind.

Turning next to the number of lepers in British territories, I have entered the main data I have been able to collect from an examination of recent Colonial medical reports and other records in Table II, and although not complete, the total number comes in round figures to 156,000, two-thirds in India, and the greater part of the remaining

third in Africa, so that when allowance is made for an equal number of early unreported cases, there must be at least 300,000 lepers in our empire, the control of which is not the least of the white man's burden.

The important question, whether leprosy is increasing or decreasing, can best be studied by an examination of the following census returns of the number of lepers in British India during the last six decades, those of the British governed territories being selected, as this area, except for the small inclusion of Upper Burma, has remained unchanged since 1881.

Census ...	1872	1881	1891	1901	1911	1921
Lepers ...	101,590	118,953	110,509	85,878	92,433	85,122
Per Mille ...	0·55	0·60	0·56	0·37	0·38	0·34

Table II

Leprosy Incidence in the British Empire

Area	Year	Lepers	Per Mille	Remarks
India	1921 census	102,513	0·32	
Ceylon	1921	577	0·13	
Malay States	1921	450	0·34	In leper institutions
British N. Borneo ...	1919	54	...	In leper institutions
Fiji	1920	450	...	In leper institutions
West Indies	1921 census	1,189	0·74	
British Guiana	1921 ,,	247	0·83	
Cyprus	1921	74	0·23	In leper institutions
Africa :—				
Nigeria	1921 census	32,000	3·2	
Tanganyika	1921	7,026	0·7	1 in every 589 of population
Kenya	1922	2,018	0·74	6 in 8,067 persons examined
Uganda	...	? 3,000	1·0	303 lepers died in 1919
Nyasaland	1921 census	1,666	1·39	Reported to be spreading
S. Rhodesia	1921	1,000	1·11	Estimated at least 1,000
S. Africa	1923	2,501	0·46	In leper institutions
Mauritius	...	600	1·6	
Palestine	1902	600	0·86	Jeanselme

Unfortunately, the striking decline in the number and the rate per mille revealed in the figures of 1901 was almost entirely due to the issue of special directions excluding cases of leucoderma and syphilis, incorrectly returned as leprosy in the first three censuses, and so is not a real decline. A small part of the decline between 1891 and 1901 was due to a series of famine years, being followed by a slight increase in 1911, while the slight fall in 1921 was probably due to the influenza epidemic of 1918-19; so we may conclude that leprosy has been fairly stationary with a slight tendency to decrease during the last sixty years, during which prophylactic measures have been practically negligible. The scare that was raised about 1890 that leprosy was increasing rapidly in India, and which led to the appointment of the Indian Leprosy Commission in 1891, is, therefore, baseless as that Commission concluded, and there are good reasons for hoping that the more effective measures against the disease now available, if only they can be fully utilised, will lead to a decline of leprosy in India in the next few decades. Although the census figures have a relative value they only include the advanced, easily recognised cases, and Dr. Muir thinks the real numbers may be as high as between 500,000 and 1,000,000 if all the early cases are included, so it may be well to point out that the first result of the more general adoption of improved methods of treatment will be an apparent increase of leprosy, due to the early and more amenable cases coming forward from their hiding places and declaring their disease, instead of hiding it as long as possible. This is already evident in the Philippines, Hawaii and elsewhere, and so far from being a real increase, it will be the first promising sign of the possibility of controlling and eventually reducing the incidence of leprosy.

The AFRICAN problem is a still more difficult one, as the real numbers in our extensive territories are quite unknown, while the poverty and the small medical staffs compared with the population and extent of the countries greatly enhance the difficulty of dealing effectively with the disease. On the other hand, the smaller number of lepers in the West Indies, Malay States, Fiji and other smaller colonies afford more hopeful fields for continued efforts to reduce the disease by the methods to be described in the next lecture, when the prophylactic measures already in force in several of our colonies will also be dealt with.

GENERAL CONDITIONS INFLUENCING THE PREVALENCE OF LEPROSY

Having now formed some idea of the prevalence of the disease throughout the world and in our empire, I next turn to a consideration of certain general conditions which materially influence the incidence and spread of leprosy, and require to be taken into account in devising prophylactic measures in different countries.

Stage of Civilization and Hygiene. One of the most potent general factors is the hygienic condition an infected race has reached ; this is well illustrated by the contrast between the conditions prevailing in Europe during the great prevalence of leprosy in the middle ages, as compared with the present time, when leprosy-infected Europeans returning from the tropics very rarely infect others under the advanced hygienic conditions in Western Europe within the temperate zone. The sanitary conditions under which the majority of the population of Great Britain dwelt during the eleventh to the fourteenth centuries cannot have been very far removed from those of the vast populations now living in one-roomed huts in India, China, Oceana and central Africa, where leprosy is now most prevalent. That such conditions are more potent than racial susceptibility to the disease is well shown by the reduction in the prevalence of leprosy, by improvement in their housing accommodation, among the large number of Scandinavian immigrants to Minnesota and neighbouring north-central states of America in the latter part of last century. Hansen himself visited the United States to trace the progress of leprosy among 170 Scandinavians, who had crossed the Atlantic from Norway when they were either already suffering from leprosy or developed the disease within the possible long incubation period after leaving their native country ; and he failed to find a single new infection in two generations, although leprosy continued to be prevalent among the same class in Norway itself. In explanation of this most interesting fact he pointed out that, whereas in their own country they had lived in such small and overcrowded houses that it was customary for all the males to sleep in one room and the females in another (as late as 1891, 42·4 per cent. of Norwegian town dwellings, according to Newsholme, having only one room), in Minnesota the immigrants built large houses, each man having his own bedroom, or at least his own bed,

thus greatly limiting the overcrowding which is such an important factor in furnishing opportunities for infection. The cool climate was also, doubtless, an important factor in addition to improved hygiene, for in the hot, humid, southern state of Louisiana, leprosy spread during the same period of last century among well-to-do French families, once more illustrating the influence of unfavourable climatic conditions. The effect of overcrowded one-roomed huts in favouring the spread of leprosy has also been pointed out by Green in Hawaii and Hearsay in Nyasaland.

Promiscuity both general and sexual, greatly favoured by overcrowding, is another most important factor in the spread of the disease, Buisson stating that the Marquesas Island epidemic was favoured by horrible sexual promiscuity ; the reports from Hawaii, where the women seldom knew who were the fathers of their children, laid great stress on the importance of the same factor, while more than one writer recorded that every European infected with leprosy was known to have had intimate relations with the native women ; Hillis reported the same occurrence in British Guiana and Drognant-Landré in Surinam and Jamaica ; Sir George Newman mentions the unrestrained sexual relationships in the middle ages in our own country as having favoured the increase of leprosy. Even in recent times in the province of Galicia in Spain, the most unusual feature of a high leprosy rate among females was attributed by Tello to their promiscuous habits during the temporary emigration of their husbands.

Other favouring social conditions, to which the spread of leprosy has been attributed in Oceana and other tropical countries with a low degree of civilization, include eating out of the same dish and smoking a common pipe, while Hansen recorded that in Norway, during the frequent visits of the socially inclined people to relatives and friends, it was considered very bad form to object to sleeping in the same bed as a leper, unless he was in a very advanced stage of the disease.

Absence of all fear of leprosy favoured the spread of leprosy in Oceana and among the fatalistically inclined Mohamedans of Central Africa, as well as in Norway seventy years ago, before segregation measures had educated the people to realise the danger ; Munch pointed out the same thing in South Russia. On the contrary, various cruel customs among savage races have been recorded as

having reduced the incidence of leprosy in Senegal, the Ivory Coast, Nigeria, Madagascar before the arrival of the French, Nyasaland, Zululand, Sumatra, India and other countries.

Spread of leprosy by immigrants has already been mentioned in tracing the infection of America and Oceana, to which may be added the following instructive modern example in British territory. In 1863, for political reasons, leprosy-infected Hottentot tribes of West Griqualand were moved right across the Orange Free State to Griqualand East to the south of Natal, with the result that they infected both the country they passed through and their new head-quarters at Kokstad, and the disease continued to spread among them until in 1895, 558 cases were known ; the neighbouring Basutos also became infected, but partly through returned miners.

The escape of tribes having no intercourse with leprosy-infected neighbours is illustrated by the absence of leprosy to this day among the more remote American-Indian tribes of Brazil, Guiana and other South American countries ; this is clearly not due to any lack of susceptibility to the disease, for Hillis recorded long ago that in British Guiana the Warara tribe, which alone had close relations with the infected negro population, including intimate relationships with some of the female inmates of the leper asylum in its early days when the administration was defective, did contract leprosy.

All the above epidemiological features of leprosy point to the disease being in some way or other a communicable one ; during the latter half of the nineteenth century a great controversy raged on the subject, in which a Committee of this College played an important, but it is now clear, an unfortunate part, due to lack of knowledge in those pre-bacteriological days. As, in spite of the lepra bacillus having been discovered just over fifty years ago, the precise manner in which the organism passes from the diseased to infect the healthy is still not completely settled, many of the facts brought forward during those controversies are still of value in the elucidation of the problem. I believe, however, that no leprologist of repute any longer doubts that the disease is a communicable one, this having been universally admitted by all recent leprosy conferences, including the third international one at Strasbourg last year.

CONTROVERSIES ON THE HEREDITARY AND CONTAGIONIST THEORIES OF LEPROSY CAUSATION

From the time of Moses right down through the middle ages, exaggerated views were held regarding the contagiousness of leprosy, doubtless due to the confusion with it of other more highly infectious skin diseases, and they resulted in the cruel segregation laws of those times. About the middle of the seventeenth century, when leprosy had nearly disappeared as an indigenous disease in Western Europe, the pendulum swung to the opposite extreme, and contagion was altogether denied ; the spread of the disease was attributed solely to heredity, just as was the case with the closely allied tuberculosis, while leprosy was also described as a ' blood dyscrasia,' and these views were still nearly universally held down to the time of Hansen's discovery of the lepra bacillus in 1873, and even after that event were maintained by some authorities, although the infective theory steadily gained ground from that moment. The most influential supporters of the hereditary theory were the great Norwegian authorities, Danielssen and Boeck, who wrote the first accurate description of the nodular and anaesthetic types of leprosy in their book of 1848, in which they supported the hereditary theory with figures relating to 213 cases ; these were for long accepted as proving their point, although when examined in the light of more recently discovered laws of heredity they have precisely the opposite bearing. The Norwegian authors regarded as proof of heredity the occurrence of two cases of leprosy within four generations of a family, even if the first case was in a grandchild and the second in a grandparent ; this method of calculation showed direct descent in 32·4 per cent. and indirect in 54·5 per cent., the remaining 10·1 per cent. being attributed to spontaneous origin, the possibility of infection in a household or family being completely ignored, while their table showed more cases in the second and fourth than in the first and third generations, as well as more in the collateral than in the direct line of descent. These data are, therefore, in reality valueless as evidence of leprosy being essentially an hereditary disease. Later Norwegian authorities, including Hansen, opposed this theory, Holmsen, for example, finding only 12 out of 93 lepers whose parents or grandparents had suffered from the disease, and

no less than 11 of the 12 had been born before their parents or grandparents were attacked, while Munro, Vandyke Carter, J. C. White of the United States, Ehlers and other authorities might be quoted to the same effect.

Still more conclusive positive evidence against the hereditary view is afforded by the children of lepers being too few to allow of leprosy surviving more than two or three generations if it were solely hereditary ; this is shown by Munro, by Lewis and Cunningham, who found only five surviving children of 52 lepers in India, and by Arning in Hawaii, where the disease spread far too rapidly to be hereditary ; by the absence of hereditary transmission among the Scandinavian lepers in Minnesota and among many hundred children of lepers brought up by missions in India and elsewhere after early separation from their leper parents ; and by the infection of numerous Europeans in the tropics, whose ancestors had been free from leprosy for many generations. Virchow thought that only a predisposition to the disease was inherited, as was so long held with regard to tuberculosis, but I have found no evidence in recent medical literature in favour of that view. Nor is the question of purely academical importance, for the anticontagionist school argued that segregation of lepers must be useless because the disease is solely hereditary in origin.

Hutchinson's Fish Theory. The late Sir Jonathan Hutchinson's fish theory may conveniently be dealt with here, as it is also of pre-bacteriological origin, dating from 1863, in its first form. It held leprosy to be ' fish-eater's gout ' dependent on excessive consumption of badly preserved fish, but modified after the discovery of the lepra bacillus to mean that a particle of badly cured fish might produce leprosy developing many years later : a difficult event to disprove, although its author later admitted that his theory would not explain the spread of leprosy among non-fish eating Basutos. This fact led him to admit in addition ' commensal communication ' through eating food other than fish contaminated by a leper ; I do not know of any surviving supporter of the theory.

THE COMMUNICABILITY OF LEPROSY

Historical. The history of the gradual re-establishment of the infectious theory of leprosy is an interesting one. In 1862, at the request of the Colonial Secretary, this College appointed a Committee to report on the infectiveness of leprosy. This Committee issued a bulky volume of evidence collected by means of a questionnaire from the Colonies and India, and concluded that leprosy was ' not contagious or communicable to healthy persons by proximity or contact with the disease,' but ' is essentially a constitutional disease indicative of a cachexia or depraved condition of the general system,' and that there was no evidence that ' would justify any measures for the compulsory segregation of lepers.' On the strength of this report the Colonial Secretary issued orders to repeal all laws in our possessions affecting the liberty of lepers (as the direct result of which, increases of leprosy have been recorded by Hillis in British Guiana, Munro in St. Kitts, Broes van Dort in the East Indies and Drognant-Landré in Surinam), although, if Vandyke Carter's statement is correct, none of the College Committee had any material experience of leprosy, and Dr. N. C. MacNamara, a distinguished Indian Medical Service Officer, came to diametrically the opposite conclusion from a careful analysis of the evidence from India published by the Committee. This unfortunate report had one good effect, namely that it stimulated leprologists with experience in leprous tropical countries, such as Drognant-Landré in 1869, Munro 1877, Brousse 1879, Hillis 1881, etc., to record convincing evidence of the communicability of leprosy. The last discordant note was sounded by the Indian Leprosy Committee of 1892, which appears to have been dominated by the ablest anti-contagionist leprologist of the day, Dr. Bevan Rake, nominated by this College, who reported that leprosy arose *de novo*, a view which was repudiated by the London Committee of the National Leprosy Fund who sent out the commission.

Evidence of the Communicability of Leprosy. The greatest difficulty in demonstrating the communicability of leprosy lies in the long incubation period, averaging several years, of the disease, which in many cases makes it impossible to trace the source of infection in endemic areas, aided by the insidious onset of the symptoms in

many cases and the tendency of patients to hide their disease. The following examples of the spread of the disease in newly infected areas, however, leave no room for doubt. In Louisiana a French woman developed leprosy in 1866, and within the next eleven years three of her four sons, one of two daughters, a nephew, an unrelated girl who nursed the mother, and an unrelated young man, who had frequently slept with the fourth infected son, all developed leprosy, although there were no other known cases in the country at the time ; the disease subsequently spread in the neighbourhood and remains to the present time. About the same time a French woman of Cape Breton Island of Western Canada, who was born opposite the Tracardie Leper Asylum of New Brunswick, developed leprosy at the age of 52, and within a few years five of her children, two of her grandchildren, a son-in-law, a man who attended the fourth infected son, and another man who was accustomed to sleep with the fourth infected son, all contracted leprosy when there were no other cases on the island ; all but the last case had died by 1881.

The carefully recorded Memel outbreak in East Prussia is equally instructive, for between 1848 and 1880 five Russian leper servant girls came to work in German families in this previously uninfected district, and by 1908 no less than 77 cases of leprosy had been traced directly or indirectly to these five original foci of infection ; the disease was still on the increase in 1899, infections nearly always occurring after long contact with a leper living in the same house. For example, four years after one of the Russian girls came to reside in a house, the father and his three children became lepers, and in a neighbouring friendly family the mother, three children, a female servant and the housewife's second husband were all attacked, and this second family infected the father, one son, two daughters, a daughter-in-law, a maid servant and two men servants of a third family, no less than 18 cases being thus traced to this one Russian leper girl.

Frequency with which Infections can be traced. In spite of all the difficulties in tracing the sources of infection of leprosy, Denny in the Philippines obtained histories of previous contact with leper relatives in 29 per cent., and McCoy in Hawaii and Gregory in Cape Colony in 37 per cent., although compulsory segregation laws made the patients loath to acknowledge infected relatives, while the percentages who acknowledged contact with lepers either relatives

or others were 60 per cent. in Dehio's South Russia cases and in Boyd and Warren's Texas series, 79·6 per cent. in Long's Basutoland, and 89 per cent. in Kereval's Caucasus cases; remarkably high figures under the circumstances, especially when it is remembered that unrecognised cases in an early stage of leprosy may sometimes be infective.

The most frequent sources and conditions of infection remain to be considered to complete this part of my subject. In this connection the relatives from whom infection is most frequently contracted may first be dealt with, the most significant facts being that in both the Philippines and Hawaii 84 to 85 per cent. of infections among relatives were those of children from parents or grandparents or from uncles or aunts, among brothers and sisters and between cousins, nearly all of whom are likely to be children and adolescents not over twenty years of age, while only 6 to 14 per cent. were older generations infected from younger ones, and the remaining 2 to 9 per cent. were conjugal infections: figures which confirm in a striking manner the evidence I shall adduce later to show that young persons are much more susceptible to leprosy than older people. Roumanian figures of infections from relatives show the same incidence, and also bring out the fact that in addition to 60 per cent. of infections of relatives, 5·5 per cent. were house infections of others such as servants, and the remaining 30 per cent. were due to other close associations.

The proportion of healthy persons living with lepers who become infected has also been investigated with very close agreement among the investigators shown by the fact that the data recorded by McCoy in Hawaii, Gregory in Cape Colony and by the Indian Leprosy Commission are respectively 4·2, 4·5, and 5·5 per cent. while in Japan and in Norway the figure in both cases was 2·7 per cent. The percentage of infections among conjugal couples are very similar, varying from 2·6 in Cape Colony, 3·8 in Japan and Norway, to 5 in Basutoland, 5·1 for males and 4·8 for females in Hawaii and 6·5 per cent. in India. Thus in both cases only about one in twenty of persons living in close association with a leper contract the disease, showing that leprosy is far from being a highly contagious disease; in fact probably not more so than tuberculosis itself under very similar conditions.

Further light on the subject is shown by a study I have made of

700 cases, in which the probable source of infection was traced and recorded in the literature of the last sixty or seventy years; an analysis of these I have already published and it may be summarised here :—

In 12·14 conjugal infection, and in 6·14 cohabitation, or 18·28 per cent. In 25·7 house infection, in 5·0 room and in 9·14 bed infection, or 39·84 per cent. Infection due to attending on a leper occurred in 19·87 per cent. Close association in 16·14, and from a leper playmate in 3·28 or 19·42 per cent. From wetnurse 1·14 per cent., wearing a leper's clothes 0·43 per cent., vaccination 0·59 per cent. and inoculation from a leper in 0·43 per cent. completes the total.

In view of the old anti-contagionist arguments that infection rarely resulted from conjugal relationship or from attending on lepers, it is interesting to note that almost one-fifth of my collected cases fell under each of these headings. Further, if the bed infections are added to those due to conjugal or cohabiting relationship nearly 30 per cent. of the infections occurred after sleeping in the same bed with a leper, while as most of the cases of attending lepers were among those nursing private cases in the patient's house, if we add a little over half of those cases due to attendance to the conjugal and house infections, we find that at least 70 per cent. of the infections took place as the result of living in the same house with a leper, usually for a considerable time : once more emphasizing the fact that long and close contact with a leper commonly precedes infection and that it is essentially a house infection, yet again bringing out a closely parallel condition with tuberculosis.

Although long and close association usually precedes infection, very short contact may suffice, three cases being on record in which young Europeans in the tropics developed leprosy within ten months to two years after cohabiting with a leper woman on a single occasion, at least one of them being under the influence of alcohol at the time. It is interesting to note that in Nigeria, Madagascar and China, native opinion firmly believes in leprosy being contracted by sexual intercourse, while lepra bacilli have been found in the semen, in lesions of the penis and vulva in lepers; the greater frequency of leprosy among males and at the age of adolescence might be explained by infection during such close relationships, although not necessarily directly as in venereal disease, as the frequency of

the discharge of innumerable lepra bacilli from the nose may be an important source of infection.

Numerous further examples of house infections might be given, the danger from leper servants for example, as shown by the Memel cases, and confirmed by Landré's 12 cases of pure European children being infected from leper servants. In well managed leper asylums the danger to attendants is almost negligible, but in the early days of the Molokai settlement of Hawaii with very defective adminstration, no less than 16·4 per cent. of infections among 244 healthy attendants were detected within three years, chiefly contracted through intimacy with the leper females. Doctors have occasionally become infected, sometimes through gross carelessness and neglect of ordinary cleanliness. Although in so many cases the probable source of infection can be traced, yet occasionally a European living under the most favourable hygienic conditions is mysteriously infected, possibly through clothes infected during washing or other unsuspected or indirect contact with a leper, such inexplicable cases in Europeans under very favourable hygienic conditions in India having come within my knowledge, so that no one can be considered quite safe while living in a leprous endemic area, however slight the risk may be.

The greater infectivity of the nodular type of leprosy is another important factor which is still too little recognised in prophylaxis, although well known to experienced leprologists. It is due to the copious discharge of lepra bacilli from ulcerated nodules and from nasal lesions in about 80 per cent., while in the anaesthetic form the bacilli cannot escape from the nerve trunks and are only discharged in the nasal mucus of 6 to 15 per cent.; in advanced chronic nerve cases the bacillary infection often dies out with loss of all infectivity, but not until permanent crippling has resulted. These cases sometimes constitute 75 per cent. of leper asylum cases in India and elsewhere, their isolation being of little prophylactic value. This difference in infectivity is well brought out by the analysis of my 700 collected cases, for in 113 of them the type of the infective case was recorded, and in no less than 94·7 per cent. they were nodular, and only the remaining 5·3 per cent., or one-twentieth, were of the anaesthetic type. In Norway special care was rightly taken to isolate as many as possible of the infective nodular cases,

while only last year the 2,501 lepers in the South African asylums were examined from this point of view and no less than 693, or 28 per cent., were released as uninfective, thus providing valuable accommodation for infective cases.

Age susceptibility of leprosy is an equally important point, which may be illustrated by the figures of over 4,000 cases in which the age at the probable date of infection, or on the first appearance of symptoms (from which the average incubation period of three to five years must be deducted), was recorded by different observers. An analysis shows that infection took place within the first 20 years of life in 50 per cent., by 25 years of age in 66 and by 30 years of age in 75 per cent., after which the susceptibility is greatly decreased.

The great susceptibility of children to leprosy is well brought out by Denny's Culion figures and the 1922 data from the same place, showing that no less than 44 per cent. and 33 per cent. respectively of the children of leper parents living with and brought up by them became infected with leprosy ; Mouritz reported 34·6 per cent. of similar infections in Hawaii, while Drs. Lie and Sand of Norway recorded that among 2,010 children of 587 couples, 7 per cent. became infected when the father alone was a leper, 14 per cent. from the mother alone, and 26 per cent. when both parents were lepers, the infection of children exposed to infection thus being five to nine times as frequent as among conjugal couples. As all the evidence goes to show that leprosy is extremely rarely, if ever, congenital, the importance of separating healthy children from their leper parents from birth cannot be exaggerated ; a case has recently been reported in which a child removed from leper parents two months after birth developed the disease three years later, although, fortunately, such early infections are very rare.

I have now completed my survey of the distribution, epidemiology and of the various factors influencing the spread and infection of leprosy, and cleared the ground for considering the mode of infection and prophylaxis of the disease in my next lecture.

Lecture II. MODE OF INFECTION AND PROPHYLAXIS OF LEPROSY

Fifty years ago Hansen discovered the lepra bacillus, which is now generally accepted as the cause of the disease. The precise manner in which the organism gains access to the human system is still unproved, owing to the failure to discover reliable methods of cultivating it or of infecting animals with the disease, and the equivocal results of attempts to infect man himself, Arning's positive result in Hawaii of inoculation of a convict having been invalidated by opportunities this man had of being infected from leprous relatives. In the case of rat leprosy, due to another acid-fast bacillus, however, Professor Marchoux has shown that the disease is inoculable, and it may be said at once that there is a remarkable consensus of opinion among experienced leprologists that human leprosy is also conveyed by inoculation of the organism through the skin or the nasal mucous membrane ; but as proof is wanting, it is necessary to consider the indirect evidence bearing on the probable mode of infection of the disease, and it was for this reason that I dealt so fully with the epidemiology of leprosy in the first lecture.

POSSIBLE MODES OF INFECTION

Inhalation has for long been regarded as the common mode of infection of pulmonary tuberculosis, although the work of Calmette has thrown grave doubt on its frequency. In leprosy the lung is very rarely affected and never primarily as far as I know, while Muir has found that the lung lesions respond to treatment more rapidly than the skin ones. There is thus no evidence that inhalation is a common mode of infection in leprosy.

Infection through the ingestion of food was thought to be possible by Hutchinson, but I can find no valid support for this view in medical literature, although it is difficult to exclude the possibility of the lepra bacillus passing through the gastro-intestinal mucous membrane and lymphatic glands to reach the blood stream without leaving any visible trace of its passage, however unlikely this hypothesis may be. Moreover, the incidence of leprosy in households rarely shows several members developing the disease at about the same

time, as in food infections, the cases being nearly always separated by a year or more, as if due to separate accidental infections by another route.

Inoculation through the skin, on the other hand, has been thought to be the common mode of infection in leprosy by such authorities as Vandyke Carter as early as 1867, Hansen and Looft, Kaposi, Munro, White, Besnier, Hillis, Mouritz in Hawaii, Tonkin of Africa and many others. The only difficulty in marshalling the evidence in favour of this view is to select from the innumerable records bearing on it, of which the following are some of the most striking.

Hillebrand reported the infection of a European child in Borneo, who thrust a thorn into his flesh immediately after a native leper boy had inserted it into his own flesh ; and Solano met with a similar case in Colombia, in which a boy aged six thrust needles into his flesh immediately after a negro leper playmate aged eight had introduced them into nodules on his limbs, with the result that the first boy soon after began to get febrile attacks and pains in his limbs, and one year later was covered with a typical tubercular leprotic eruption. These two cases are of especial significance in view of the subjects being children of a highly susceptible age, whereas all the negative experimental inoculations I have found recorded were in far less susceptible adults, none of those inoculated by Profeta having been under 25, while the majority were over 30 years of age, when susceptibility is small.

Even more conclusive are the following two cases of doctors inoculated while operating on lepers. Dr. Ehlers reported the case of a Danish doctor in the West Indies, who wounded a finger during an obstetrical operation on a leper negress ; the wound healed slowly and he soon after developed in the injured finger severe pains, which later proved to be due to anaesthetic leprosy ; in Hundadze's case a medical man inoculated a wound of his right finger in opening a leper's abscess ; the wound healed normally, but two months after the site of the wound became red and swollen, this symptom being accompanied by fever for three days, and three weeks later red leprous patches appeared on the injured finger ; these were at first taken to be syphilis, but proved to be leprosy, leaving no doubt of infection through the wound. Hatch in Bombay has recorded a case of anaesthetic leprosy of the ulnar nerve closely

following a wound made while doing a post mortem on a typical leper, the diagnosis being confirmed by Vandyke Carter.

These cases, among others which might be quoted, should suffice to establish the possibility of leprosy being inoculated through the skin, while the close resemblance between limited dermal leprosy and tubercular dermatitis, now generally accepted as due to inoculation with the tubercle bacillus, presents an interesting analogy in a closely allied organism.

Vaccination as a possible cause of leprosy has given rise to much controversy and some loose statements, but very suggestive facts are on record ; the possibility of its occurrence receives strong support from Arning's observations in Hawaii, proving that lepra bacilli were frequently present in vaccine lymph taken from apparently healthy skin in persons with extensive cutaneous leprosy, but not in vaccine from purely nerve cases, and he considered that there was strong evidence that leprosy had been spread in certain parts of Hawaii by carelessly performed arm to arm vaccination. Cases highly suggestive of infection in this manner have been recorded by Hillis, Gairdner of Glasgow and others, but as they occurred in endemic areas other modes of infection cannot be excluded ; it is, however, of interest to note that Gavin Milroy records that Creole families of the West Indies obtained their lymph from the United States for fear of leprosy. Quite recently new light has been thrown on the subject by the observations of O. E. Denny and R. Hopkins at the United States Leprosarium, where after vaccination of both the healthy staff and the lepers, the latter alone had severe reactions ; in eleven of them local swelling, fever and the appearance of fresh nodules and even ulnar neuritis appeared ; so that it is possible that such reactions in early unrecognised or latent cases of leprosy may formerly, in Hawaii and elsewhere, have been taken for infections due to vaccination. In any case, infections clearly due to vaccination in leprous countries by the old arm to arm method can have very rarely resulted, while they are impossible with the general use of calf lymph at the present time.

Infection from clothes and bedding has frequently been suspected, Tonkin regarding this as a common occurrence in badly infected Central Africa from Nigeria to the Sudan, where it is the custom for clothes to be passed down, without ever being washed, from the

rich to the poorest until worn out ; to the sleeping of whole families together on mats in Hawaii, India and elsewhere have been attributed inoculations over the buttocks, shoulder-blades and cheeks ; a case of a primary lesion in the last position in a child, who slept on the bed of a leper relation, having recently been reported by Muir in Calcutta ; wearing lepers' clothes has also been recorded as a cause of infection in the West Indies and Norway, etc., while in Riga a large proportion of the few lepers occurred among washerwomen : all indicating possible infection through inoculation from clothes or bedding soiled with leprous discharges containing enormous numbers of bacilli.

Sites of primary lesions as evidence of inoculation afford further important evidence, the especial frequency of anaesthetic leprosy in bare-footed races having been recorded in Abyssinia, where ten out of twelve lepers are said to be of this type, the Sudan, Hawaii, Crete, Java (with 50 per cent. of such cases according to Gell), Peru and India. Only last year Muir published diagrams of the distribution on the surface of the body of the first noticed lesion (most of which are considered to be the probable primary sites of inoculation), of no less than 1,056 lepers in Indian asylums and noted that they greatly predominated on exposed parts, such as the face, and extensor surfaces of the extremities, as well as over the buttocks and shoulder-blades exposed to friction on bedding and sleeping mats. Further, in two areas on alluvial soil, primary lesions on the feet were very uncommon, but in two separate hilly and stony districts among the bare-footed they were frequent on the feet ; all facts indicating inoculation through the skin as the most usual mode of infection.

Inoculation through the nasal mucous membrane is another possible mode of infection first pointed out by Professor Jeanselme in 1897 and supported by Stricker, Muir and others, but recent examinations of a number of children of lepers in the Philippines for early signs of the disease showed none in which the nasal mucous membrane was infected without skin lesions, although the opposite occurrence was common, so that primary nasal infection is probably much less frequent than that through the skin.

The rôle of insects in the transmission of leprosy has received much investigation in recent years without any one insect being especially incriminated. Flies can carry numerous lepra bacilli

from ulcerated skin lesions to the skins of neighbours or to food, indicating the advisability of putting a stop to markets being frequented by begging lepers as is so frequent in the East. Acid-fast lepra-like bacilli have also been detected in the alimentary canals or faeces of insects fed on lepers, as shown by the following recorded data :—Bed bugs have yielded positive results in 9·9 per cent. of 302 bugs fed on lepers, and in one per cent. of those caught on them, while E. C. Long has recorded the development of leprosy in a man in South Africa within a year of his having slept in a bug-infested hut recently vacated by a leper, although he had never lived in a house with one. With a single exception investigators have found under one per cent. of mosquitos fed on lepers to harbour the bacilli. The *Acarus scabiei* of itch has been suspected by Heiser and by Mugliston to play a part in leprosy infection, while lice have also been accused.

Explanation of Relation between Humidity and Leprosy. I may now return to the relation between high humidity and temperature and leprosy rates which I showed in the first lecture, and for which I have suggested the following explanation. In the first place, as the leprosy bacillus has not yet been cultivated with certainty, it presumably lives but a short time outside the human body in the absence of any known animal or insect carrier, so that the absence of leprosy in very dry, hot countries may be explained by those conditions being most unfavourable for the extracorporeal survival of the leprosy bacillus; while in hot, damp climates it will find more favourable conditions, especially if it gets on to the skins of neighbours of an infective leper. Such climates also greatly favour insect life, while the frequency of the first noticed lesions on the exposed parts of the body and extremities is easily explained on the ground that they are subject to frequent insect bites, affording minute lesions in the skin through which the lepra bacilli may gain access to the dermal connective tissues and nerve endings so favourable for their multiplication; the presence of humidity and numerous insects thus favour infections from lepers living in the same house or in close association with the healthy, these being, as I showed in the last lecture, the essential conditions under which the disease spreads. Such an explanation also affords a good basis for the study of prophylactic measures, to which I must now turn.

THE PROPHYLAXIS OF LEPROSY

Hitherto, the prophylaxis of leprosy has practically been summed up in the one word segregation. The first isolation laws are probably those of Moses; drastic measures were enforced in the middle ages in Europe, including deprivation of civil rights, divorce, isolation in leper houses, wearing a special garb, prohibition of entry into inns, churches and bakehouses, touching or giving anything to children, washing in common fountains, eating or drinking with any but a leper, speaking in the streets except in a whisper, etc.; all highly effective measures against a disease now known to be rarely acquired except by prolonged close contact with a leper. It is not of the highly contagious nature it was thought to be in ancient times and by some savage races to-day, who are recorded to have tried to control leprosy by killing all young lepers in Nigeria, or all ulcerated ones as in Nyasaland and Fiji, burning them alive in Sumatra, drowning them in Western India, etc. Isolation of advanced lepers in special villages has been reported from Central Africa, Madagascar, Indo-China, China and parts of India such as the Garo Hills in Assam, while even in Spain lepers are said to be driven into the mountains and deserts: so great is the fear of this mutilating and disfiguring disease, which still allows of severe segregation measures necessitating life-long isolation in most cases, being sanctioned by public opinion, such as would not be tolerated against other diseases with such a protracted course. The whole policy of segregation against leprosy requires careful reconsideration in the light of our present knowledge of its epidemiology, as a preliminary to which the results hitherto obtained by the measure must first be reviewed, beginning with those under the most favourable conditions in European races in the temperate zone.

The Norwegian System. As the greatest obstacle to segregation is the natural tendency of the sufferers to hide their affliction, the essence of the humane Norway system of segregation is to provide comfortable hospital conditions to attract the lepers, and to resort to compulsion as little as possible, that measure only being applied to indigents during the early years. In 1856, when the measures were commenced, there was good reason to believe that leprosy was increasing in the country, 2,833 cases then existing, or 1·91 per mille, six times the present official rate in India, and

only 235, or 8·3 per cent. were in leper institutions. Careful records were kept, while newly discovered cases were entered in the year in which their symptoms appeared, and after a time Hansen and Looft were able to report that the decrease in different districts was in proportion to the numbers isolated, and that the new cases arising in any area were in proportion to the number of unisolated nodular infective cases forming foci of infection. Owing to the favourable results obtained, the laws were strengthened in 1878 and again in 1885, when all lepers were isolated at public expense, and local authorities had power to remove lepers from their houses, unless they could be isolated at home under satisfactory conditions and close supervision. The results are shown in Fig. 1, which I have

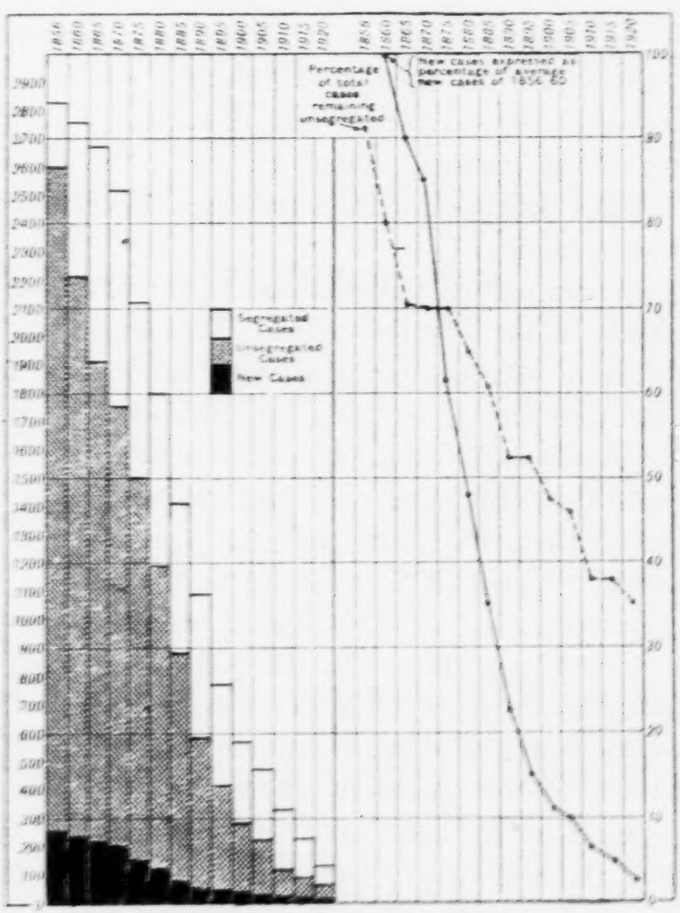

Fig. 1. Chart illustrating segregation in Norway.

prepared from the official figures, the latest having been kindly supplied to me by Dr. H. P. Lie (who succeeded Dr. Hansen in

charge of the work), the average number of new cases, and of the segregated and unsegregated being shown for each five-yearly period, while to the left the curves of the percentage of new cases, as compared with the average number from 1856-1860, and the percentage of unsegregated lepers in each five years are also given, showing a reduction of the total cases to 160 in 1920, while in 1923 there were only 140, or 5 per cent. of the original number in 1916-20 ; the new cases were only 2·7 per cent. of those in 1856-60, although it was not until after 1890 that over half the total lepers were isolated, these cases, however, including a much larger proportion of the infective nodular types. An interesting feature of the Norway experience was that five out of six of the newly-discovered cases dated their first symptoms back for three or more years, during which they had opportunities of infecting their households before they were discovered and isolated (a difficulty which is still greater among backward tropical races), and it accounts for the slow, if sure, effects of the measure, which is one of the most successful yet recorded, and clearly establishes the value of efficient segregation where it is practicable.

In SWEDEN compulsory notification and the provision of hospital accommodation in the affected central Helsingland and Dalecarlia districts led to a fall from the maximum number of 103 cases in 1873 to 89 in 1907 and 37 by 1923, a reduction of 65 per cent., the provision of comfortable conditions in the leper institution enabling a high proportion of the cases to be isolated without compulsion.

In ICELAND there were 226 cases, or 3 per mille in 1896 ; the leper hospitals, which had been closed in 1848 on account of the non-contagionist views of Danielssen, were reopened through Dr. Ehlers' influence in 1897, who also got a law passed prohibiting children under fifteen years living in the same house with a leper—a measure which would be of great value in this country if it could be applied to open infectious cases of tuberculosis. The result was that leprosy declined to 67 cases and 0·78 per mille by 1920, or just one-fourth of the rate twenty-four years before, fully justifying the steps taken. I am indebted to Professor Monrad-Krohn of Christiania for the recent figures of these three countries.

In MEMEL compulsory segregation and search for contacts were enforced in 1893, when there were 25 cases and the disease was still

increasing ; a decrease set in in 1898, by 1908 only 15 controlled cases remained, and by 1914 the outbreak had been stamped out : these results confirm the value of humanely conducted segregation in European countries. Further, in the New Brunswick province of Eastern Canada an old endemic focus dating from 1815 among the French was controlled, with the aid of the Catholic priests, by a voluntary system of isolation, and the cases slowly decreased from a yearly average of 32 in 1875-79 to 20 in 1910-14, and with more active measures fell to 12 in 1920-22, including a few from other parts of Canada.

Segregation in the Sub-Tropical Zone, 40° to 23½° F. In countries of this zone with moderate temperature and rainfall, leprosy rates are mostly low. CYPRUS in 1878 had over 150 known lepers ; in 1891, compulsory notification and segregation were introduced ; in 1901, there were 125 cases, 0·57 per mille, and in 1921 only 74, or 0·23 per mille, a decline of 60 per cent. The 1921 report states that there is now ' a good prospect of stamping out the disease in Cyprus before very long.'

The AUSTRALIAN COMMONWEALTH mostly falls in this zone, except the north of Queensland and the little-inhabited north coast, and compulsory segregation has been enforced for some time ; leprous Chinese and Oceanic islanders are repatriated, Europeans being retained until bacteriologically free for several years. Queensland had 78 lepers in 1910 and 50 in 1921, while New South Wales has had from 24 to 20 with a yearly average admission of three during the last fifteen years up to 1922 ; the remaining colonies have only isolated cases. A single institution might well care for all the cases in the Commonwealth.

SOUTH AFRICA presents a much more difficult problem with its large native population, and records of the admissions to the Robben Island settlement since 1845 show 20 to 30 yearly up to 1886, while immediately after the introduction of compulsory segregation in 1892 the yearly admissions rose to 294, and averaged 107 from 1894 to 1905 ; as late as 1907, Mackie reported that only 23 per cent. of the lepers registered that year could be accommodated in the asylums, and the recorded rates per mille for Cape Colony rose from 0·41 in 1891 and 1·02 in 1895 to 2·21 in 1907, clearly showing that in the absence of efficiently enforced segregation the disease was slowly increasing.

Recently the Union of South Africa has provided additional accommodation, and in 1923 there were 2,501 lepers isolated in several asylums, including the 693 bacteriologically negative cases since released as uninfective. In Basutoland, Long reports that the spread of the disease has been controlled by the active segregation measures enforced there in recent years.

Segregation in Tropical Countries. Still greater difficulties are met with in humid tropical countries with backward populations, although in some restricted areas with small numbers of lepers, promising results have occasionally been obtained; these are best illustrated by the WEST INDIAN data I recently collected from the colonial reports, which are of especial interest,—the colonies who did not carry out segregation furnishing admirable controls, as shown by the following data:—

Jamaica began segregation in 1896. In 1891, there were 450 lepers, 1·23 per mille, and in 1921 only 319, 0·35 per mille, a decrease in twenty years of 52 per cent.

British Guiana began segregation in 1905. In 1891 there were 353 lepers, 1·23 per mille, and in 1921, 247, 0·83 per mille, a decrease of 42 per cent.

Trinidad only began segregation in 1915. Lepers in 1871 numbered 102, 0·93 per mille; in 1891, 225, 1·12, and in 1921, 526, 1·50, an increase of 81 per cent.

Barbadoes had no segregation up to 1922. Lepers in 1901, 230, or 1·21 per mille, and in 1921, 164, 1·05 per mille, an increase of 48 per cent.

St. Kitts, with no segregation, had 112 lepers, 2·44 per mille in 1891 and 100, 2·60 per mille.

Of the smaller islands St. Lucia and Granada, without segregation, showed no change in the leprosy rate per mille, while St. Vincent, with segregation of indigent lepers, showed a decrease from 57 in 1881 to 30 in 1911 and 19 in 1921.

Thus all the colonies who relied on voluntary isolation in asylums of a few advanced cases showed either an increasing number of lepers or a stationary condition, while the island colonies which carried out compulsory segregation showed a great reduction within two or three decades; thus affording a striking object lesson to other colonies.

FIJI commenced compulsory segregation in 1911 on an island settlement, where the numbers isolated increased from 40 in 1911 to 351 in 1920, and are now being treated efficiently by Dr. Harper, but it is too early to judge of the effects of these measures. Penrhyn Island has partial segregation, and only 27 cases in seventeen years. In the MALAY STATES a Leprosy Prevention Ordinance was passed in 1893 and leper asylums were gradually opened; the average yearly admission increased steadily from 150 in 1895-1901 to 174 in 1912-16, but fell to 152 in the five years up to 1921, indicating that a decline of the disease has now set in. CEYLON passed a leper ordinance in 1901, but deficient accommodation has prevented its efficient enforcement.

In our TROPICAL AFRICAN COLONIES, lack of funds has prevented anything material being done, except in Tanganyika, where there are 39 leper villages with land to cultivate, making many of them self-supporting.

In MADAGASCAR many lepers are isolated in settlements under missionary care, but I have not been able to get any information on the results yet obtained.

Two important trials of segregation in backward tropical areas under most difficult conditions remain to be considered, as they have important lessons.

In HAWAII, as early as 1865, a leper hospital was opened near Honolulu for the treatment of the earlier cases, and the famous leper settlement where Father Damien fell a victim to the disease after many years of invaluable work, was formed on the Molokai peninsula; but in spite of an expenditure of 4 per cent. of the total Island revenues the conditions were bad, resulting in the inevitable hiding of cases as long as possible, so that over 90 per cent. were of several years' standing when first discovered. Moreover, political influences caused great variations in the enforcement of the law, and very little effect was produced on the prevalence of leprosy, until in 1891, American influence greatly improved the conditions, since which the rate per mille has fallen from 13·5 in 1890 to 2·16 in 1919; during the last few years effective treatment has been introduced with favourable results, which I shall deal with in the next lecture.

In the PHILIPPINES the Americans, under Dr. Victor G. Heiser, inaugurated, under difficulties, a great effort by means of compulsory

segregation to stamp out leprosy, and opened the Culion settlement in 1906, regarding the results of which contradictory statements have been published. Thanks to the kindness of Dr. H. W. Wade, chief medical officer, in supplying me with the official figures to date, I have been able to work out Fig. 2, the upper curve of which shows the yearly (continued line) and three-yearly (broken line) average admissions, while the lower curve shows the yearly total Culion lepers (continued line) and the average three-yearly number corrected to the average mortality of the whole period (broken line).

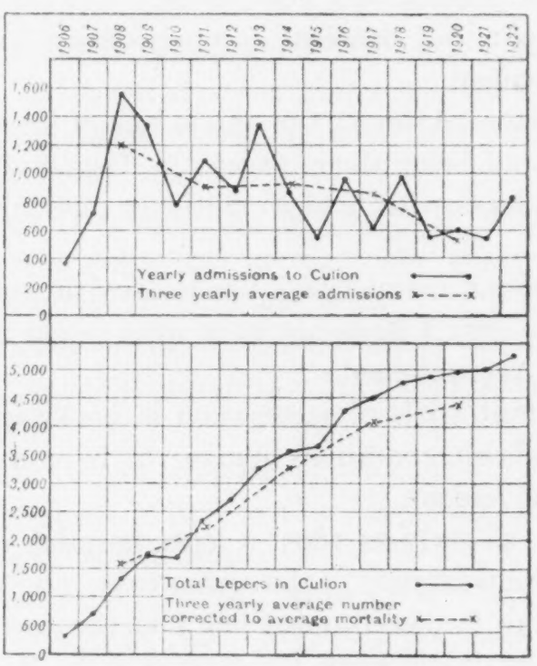

FIG. 2. Chart illustrating the result of segregation in the Philippines.

The admissions naturally show the highest rate in the first three years after the opening of the settlement, the second three years show a steady rate, the third a slight fall, and the three years 1919-21 a marked fall in the admissions, while 1922 shows a rise due to the satisfactory reason that numerous lepers voluntarily declared themselves to obtain the benefit of the improved treatment now available. The lower curve shows that the total number in the colony is still rising, although at a gradually less rapid rate, the flattening-out of the curve being especially marked for the years 1918-21, indicating that the peak of the curve was nearly reached, apart from the new factor introduced by the attractive power of the

new treatment. In view of the comparatively short duration of the measure, and the immense difficulties in collecting the lepers from the numerous islands of the Philippine group, this result cannot be considered unsatisfactory, although not coming up to the sanguine expectations with which it was started, and with the assistance of the American doctors now applying the improved treatment, still better results are likely to accrue within the next decade or two.

Failures of Segregation and their causes. In addition to the foregoing examples, many failures of attempted segregation are on record, which have their lessons, and they may most conveniently be classified in accordance with the causes of their lack of success.

Perhaps the most common cause of failure is vacillating policy, such as in French Guiana, where betweeen 1823 and 1891 no less than twenty administrative alterations in policy were promulgated, rendering continuous application of efficient measures impossible; Hillis and Neal record very similar conditions in British Guiana. In New Caledonia, the French first ineffectively tried isolation of the lepers in five villages, later in a central asylum on Art Island, which had to be given up for financial reasons, and then reverted to forty-two small leper villages, which failed owing to lack of authority of the native chiefs, although in the Marquesas Isles this plan is said to have been successful with the aid of gendarmes. Political influences prevented effective enforcement of leper laws in Louisiana and Algeria as well as at first in Hawaii.

Defective administration has been another frequent cause of failure, the worst instance of which is probably that of Crete, where Ehlers showed that under Turkish authorities the leper occupants of the four leper villages outside the four main towns not only begged in the cities, but the more successful of them bought their houses in the leper villages, and then let them to healthy persons while they went on begging tours through the island, and some of those who took their houses contracted leprosy; the so-called segregation being in reality an ideal method for spreading the disease: yet Hutchinson quoted this as an example of the failure of segregation. Deficient accommodation, such as obtained a few years ago in South Africa, also comes under this heading.

These examples will suffice to show the great difficulties encountered in enforcing efficient segregation in many tropical countries,

mainly due to the impossibility of discovering and isolating the earlier cases as long as we have no effective treatment of the disease. Moreover, the numbers of lepers in China, India and Africa make general segregation quite impracticable at the present time, if only on account of the utterly prohibitive expense, so the successful Norwegian system cannot be applied to any large proportion of the world's lepers without the addition of some means of attracting the early cases and of dealing with them by some simpler, cheaper, and more effective method than life-long isolation.

Improved treatment as an aid to prophylaxis is the essential requirement, and in order to enable me to suggest a policy for reducing the incidence of leprosy I must here anticipate the main conclusions I shall come to in my next lecture regarding the present position of the improved treatment, which will then be fully dealt with, and may be summarised in the following statements :—

1. Early cases of leprosy are now coming forward voluntarily for the new treatment in Korea, the Philippines, Hawaii, India, and elsewhere in numbers formerly unknown.

2. Muir has now shown that the majority of these early cases are not infective, so may safely be treated in out-patient clinics at small cost, and that nearly all early cases lose their symptoms within a few months—the few bacteriological positive ones become negative and consequently no longer a danger to their households, while a fair proportion of later cases also clear up under prolonged treatment.

3. When a considerable proportion of the early cases can be thus cleared up, and members of their households examined repeatedly to detect and treat, on its first appearance, those developing the disease, the number of foci of infection in the houses will rapidly be reduced and the occurrence of new cases and the incidence of the disease will steadily fall.

By combining this new and hopeful measure with the isolation of as many as possible of the more advanced infective cases in special hospitals, sanatoria on the lines of tuberculosis hospitals, and colonies with land to cultivate and with the provision of the best treatment, a far more rapid decrease of leprosy will be brought about than ever previously possible. The Honolulu results of the last three years, which I shall deal with in my next lecture, indicate that, if they are maintained for another seven years, the total number of known

lepers in those islands will be reduced by nearly one-half within a single decade, or three times as rapidly as under the successful segregation system in Norway.

THE PRINCIPLES OF MODERN LEPROSY PROPHYLAXIS

The best prophylactic measures to adopt, necessarily vary widely under the conditions prevailing in different countries and climates, so that only the general principles can be laid down ; these can most conveniently be considered first as regards areas in which compulsory segregation is adopted with a view to rapid reduction of the disease, and secondly, as regards the measures which are possible where compulsory methods are impracticable.

Legalised Compulsory Measures Should Include :—

1. Compulsory notification of lepers by both medical men and laymen under penalties, as recommended by the Paris Academy of Medicine in 1914, and enforced in Sweden, Iceland, the United States and some of our West Indian Colonies.

2. Examination and admission to institutions of the lepers by an expert medical board, which should also deal with cases recommended by the institution authorities for release as no longer infective, as in Honolulu and the Carville leper colony of the United States.

3. Compulsory isolation, with the provision of the best treatment available, as laid down in a resolution of the 1923 Strasbourg International Leprosy Congress, and retention until the patient is proved by repeated bacteriological examinations to be free from all infectivity, any released cases being examined at fixed intervals for several years, to detect and deal with possible relapses, as in the United States, Hawaii and the Philippines.

4. The examination of all household contacts of discovered lepers, about every six months for from three to five years, to detect new infections in the earliest and most curable stage of the disease. These periods are based on the results I have recorded of a study of 84 collected cases indicating an average incubation period of two to three years, while in 65 per cent. the time did not exceed three years and in 81 per cent. was not over five years ; these figures show that four-fifths of new infections would be detected within the longer period.

5. The separation of the children of lepers from their parents as soon after birth as possible, and the prohibition of healthy children and adolescents from living in the same house with a leper, as in Iceland, where an unusually rapid decrease of leprosy has recently taken place. The prohibition of leper children or teachers in schools for healthy children is also essential, but has frequently been neglected.

6. The separation of the sexes in leper institutions, except husbands and wives beyond the child-bearing period, as enforced in Australia and the United States, the neglect of which measure in Hawaii and formerly in the Philippines, as already mentioned, led to infection of a large proportion of the children born to the lepers. In Panama an effective compromise has been adopted, worthy of imitation where complete separation of the sexes has not been found practicable, by only permitting marriages when the male leper applies for and submits to the simple operation of sterilisation by bilateral section of the vas deferens, which does not affect marital relationship, while preventing the procreation of children. The Calcutta Leprosy Conference of 1921 urged separation of the sexes to prevent the calamity of a healthy partner becoming infected while the other was recovering under treatment.

7. Countries adopting such stringent measures to eradicate leprosy must have legal powers to protect themselves from re-infection by repatriating all immigrants arriving with or developing leprosy within the longest known incubation period, which in exceptional cases has been known to extend to twenty or more years.

8. The prohibition of lepers from engaging in dangerous occupations, such as the preparation or sale of foods, clothing, cigars or cigarettes, the care of children or sick, domestic employment, midwifery, the barber's trade, prostitution, etc., is essential in all leprous countries, although not yet enforced even in India.

Home isolation was permitted in Norway, mainly in the less infective nerve forms, where the accommodation permitted the leper being provided with separate room, cooking and table service, bedding, clothing, washing, etc., under close medical supervision; but this measure failed to prevent infections in Roumania and South Africa, and should not, in my opinion, be permitted when

there are any children in the house or when the case is an infective nodular one.

The treatment of early uninfective cases, in which no lepra bacilli can be found by repeated examination in the skin or nasal mucous membrane, as out-patients in special hospital or dispensary clinics, which Muir is rightly advocating as the most practical measure in such countries as India, where compulsory segregation is impossible, may also have to be considered where compulsory methods are enforced, and this measure appears to be worthy of trial to attract the early more amenable cases, with the proviso that any patients not regularly attending until released by an experienced medical officer will be liable to be segregated.

Prophylaxis where Compulsory Segregation is Impracticable. It will be remembered that in the early years of the Norwegian work, and throughout the successful campaign in Sweden, many lepers were isolated on a voluntary basis by supplying good hospital accommodation for them. Now that a far more effective treatment is already attracting many earlier cases of leprosy to hospitals, much more can be done towards isolating lepers on a voluntary basis than formerly, and the measures best suited to extend this policy remain to be considered; the following system has been advised by the Calcutta Conference and endorsed by the Government of India, and is gradually being brought into operation in the different provinces as funds permit.

Leper Asylums and Colonies in India. At present there are some 9,000 lepers segregated in India, some of the large cities having prison-like asylums with high walls surrounding small spaces, but most of the cases are in country asylums administered by the Mission to Lepers and other missionary bodies, with financial assistance from the Government, ranging from the well-organised Purulia institution with 700 lepers and ample ground for cultivation, to a small house with ten or so lepers. Some three-fourths of the inmates are advanced anaesthetic mutilated cases, for the most part uninfective and quite unamenable to treatment, whose isolation has little or no prophylactic value, while, unfortunately, in many of the asylums regular up-to-date treatment is not yet available.

Under the new system now being introduced, large institutions are being provided with ample land for cultivation, work on which

is as beneficial to lepers as exercise is for the tuberculous, and will also enable most of the required food to be home grown. Only infective cases should be admitted, under an efficient administrative and medical staff providing the best treatment and care, hospital accommodation for advanced and complicated cases, and a separate area, on a cottage or other convenient system, for the earlier ones to save them being repelled by the mutilated class, and another separate part for healthy children, the sexes being separated on the lines already mentioned. Patients becoming bacteriologically negative should be kept in a convalescent section until passed for discharge as at Carville, or separate small colonies with land provided for them as at Purulia, where there is already a village of recovered lepers, something on the lines of the Papworth tuberculosis colony near Cambridge.

These leper colonies, or, as they might well be called, sanatoria, will be complementary to the hospital and dispensary clinics already mentioned, as any advanced cases unsuitable for treatment at the latter, and likely to repel the attendance of the early amenable class it is so important to attract, should be sent to the colonies for prolonged treatment and care. Where the clinics are opened before colonies are available, the difficulty may be dealt with by arranging for the early cases to attend on different days from the more advanced and infective ones.

As the proposed well-equipped and staffed colonies will be expensive institutions to maintain, I regard it as most essential that advanced mutilated, little or not infective, begging anaesthetic cases should not be admitted to them, as treatment will be useless, and its apparent failure depressing to the more amenable nodular cases, while it is a waste of good money to isolate cases who are no danger to the community, and should be looked after by their relatives. The good example of South Africa in releasing from the asylums, the 693, or 28 per cent. of the total of such uninfective lepers is worthy of imitation in other countries without unlimited funds for dealing with leprosy.

In our tropical African colonies the difficulties of dealing with the leprosy problem are even greater than in India, although the same principles are applicable, beginning with the provision of leprosy clinics for treatment until the confidence of the patients is gained,

when the more infective cases may be induced to live in leper villages, which are already a familiar measure among many tribes. Sir Hugh Clifford, in Nigeria, has started some leper villages into which only lepers are admitted, allowing them to retain their self-respect, while all children born to them are removed from danger of infection by being sent to healthy relatives as soon after birth as possible : an admirable system which is worthy of wide adoption under similar circumstances.

Such, in brief outline, are the conclusions I have come to on the vexed question of leprosy prophylaxis based on three years' minute study of the literature, and adapted to take full advantage of all the recent advances in our knowledge of the epidemiology and treatment of this terrible disease. Many decades of patient work lie before us, but I feel it is high time we made a serious attempt to utilise the means we now possess of reducing leprosy in our Empire ; regarding which our American cousins have recently set us such a good example in Hawaii and the Philippines.

Lecture III. THE TREATMENT OF LEPROSY

Preliminary Considerations. Leprosy presents many difficulties in estimating the effects of any given treatment, due to differences in the types of the disease, the very chronic and variable course it runs with sudden exarcerbations, sometimes followed by temporary improvement. Moreover, in the anaesthetic variety there is a tendency for the progress of the disease to cease and even for the infection to die out, although rarely until after serious permanent crippling of the extremities has been produced through nerve destruction rendering treatment ineffective. On the other hand, when ill-nourished begging lepers are cared for in an asylum, considerable improvement may ensue without drug treatment ; for which reasons short trials of drugs in a few cases have little value. Even more difficult is the estimation whether great improvement, even amounting to disappearance of all active signs and infectivity of the disease under prolonged treatment, will prove permanent or not : the analogy with tuberculosis being here very close, for it is impossible to say whether any living bacilli which may produce a relapse some years later remain quiescent in the tissues. The spontaneous

disappearance of very limited anaesthetic patches has also very occasionally been met with, but natural recoveries of the nodular forms rarely if ever occur, although such cases may sometimes develop nerve symptoms, which gradually predominate over the skin ones, forming mixed cases of leprosy. In short, there is a nicely balanced struggle between the invading bacilli and the tissues of their host, liable occasionally to be turned in favour of the latter, which necessitates caution in judging of the effects of any remedy without long observation on a series of cases, but which also affords good hope of curative measures being discovered by patient research. These now appear to be within sight in certain soluble derivatives of chaulmoogra and other oils, before dealing with which a brief review of other methods which have afforded great temporary improvement and a few apparent cures will be of interest and throw some light on the conditions favouring recovery.

Mineral Preparations. Mercurial preparations have been recommended from the days of Pjetursson in Iceland, in 1769, to Radcliffe Crocker in 1896, but have not recently met with favour. Arsenic was advised by Danielssen in Norway, while atoxyl, arrhenal, salvarsan and, recently, eparsono have been advocated, chiefly by French writers, with variable and uncertain results. Antimony intravenously has recently been advised by F. W. Cawston, and appears to be of some value in healing leprotic ulcers, although workers in Culion and elsewhere have not been able to confirm the original claims made for this form of medication. Cyanocuprol has been advised in both tuberculosis and leprosy by Japanese workers.

Iodine has greater claims; Danielssen in 1886, and others, using the iodide in the treatment and diagnosis of leprosy, observed febrile and local reactions in nodular lesions, with the disappearance of old and the appearance of new nodules, as well as increased discharge of lepra bacilli in the nasal mucus of diagnostic value following its use. Iodoform and europhene injections have been advised by Neisser and others, while Clegg and Hollmann obtained interesting febrile reactions after the inhalation of 15 to 30 minims of ethyl iodide, and Marchoux and Bourret in 1909 observed that during reactions following iodides, large numbers of leprosy bacilli lose their acid-fastness and are destroyed; so the drug may be of

value in conjunction with other remedies, although by itself it has failed to produce lasting beneficial results in leprosy. Ichthyol, guiacol, strychnine, etc., have also had their advocates.

Local treatment has been advocated, especially by G. Unna, including the destroying of nodules by shaving off with a razor, applying carbolic acid, hydrochloric acid, caustic potash, the cautery, etc., but the claims to eradicate the disease in early cases by such measures have not been substantiated. Tincture of iodine, carbon dioxide snow, ethyl chloride (Lie), trichloracetic acid, mineral baths, X-rays, radium and electric currents have all had their advocates: the multiplicity of remedies indicating that no really satisfactory one was available up to very recent times.

Serums against leprosy have been prepared by Carrasquilla in Colombia, in 1896, by injecting horses with the blood of lepers, while later others, with more reason, but without success, injected animals with antigens composed of juice of nodules, containing lepra bacilli while Dyer in America tried antivenomous and normal horse sera with no result.

Vaccines made from various acid-fast organisms have been extensively tried with temporary good results in some cases. Tuberculin produces well-marked reactions in leprosy, but with large doses of Koch's original form more harm than good was done, an analysis of fourteen papers up to 1892 showing that slight improvement was only claimed in one trial, but in 1896 Arnaud saw disappearance of nodules and improvement continuing for two years following a severe reaction: a case that once more illustrates the great benefit occasionally following upon violent reactions induced by very different lines of treatment. In 1904-5 Lie of Norway recorded post-mortem evidence to prove that reactions may be obtained with tuberculin injections in lepers who were quite free from lesions due to the tubercle bacillus, but he failed to get benefit in lepers he treated with small doses of tuberculin, although in 1909 Baber reported remarkable improvement in several cases after the use of tuberculin combined with chaulmoogra oil.

Nastin is essentially another non-specific vaccine made by Deycke by dissolving a fatty substance, extracted by ether from an acid-fast streptothrix, in benzoyl chloride, the injection of which, in leprosy, also produced febrile and local reactions, sometimes

followed by considerable improvement, very promising results being reported for a time by Deycke and others, until a four years' trial in British Guiana, initiated by the discoverer himself, and reported on by Wise and Minett in 1912, showed that general reactions with softening and absorption of the nodules only occurred in 3·5 per cent., of the cases treated and in the remainder early slight general improvement during the first three months was followed by retrogression, and the patients got steadily worse ; their conclusion from prolonged study of 244 unselected cases was that nastin produced only ' a slight temporary check during the first six months of treatment, but otherwise the natural course of leprosy continued unchanged.' Such temporary changes accounted for the improvements shown in Deycke's tables, while subsequently Minett found that injections of benzoyl chloride alone produced precisely the same effects as Deycke's nastin-B itself.

Vaccines from supposed acid-fast bacilli of leprosy have been made by Rost, Williams, Clegg and others from cultures obtained from cases of leprosy, which, however, Walker has recently shown cannot be distinguished from the smegma bacillus, so are also non-specific acid-fast bacilli, but their use has undoubtedly been followed by local and general reactions, as with tuberculin, followed in some cases by great improvement, only too often of a temporary nature. Harm, however, can also result ; Rutherford, in twenty carefully noted cases, found that the deterioration exceeded the improvement, while Clegg's bacillus gave negative results in Honolulu : the effects of this treatment on the whole are, therefore, disappointing.

Vaccines made from excised lepra nodules, containing enormous numbers of lepra bacilli, have also produced some benefit, but this plan has obvious limitations, especially where the disease is not common. Nevertheless, as will appear presently, the reactions produced by the various acid-fast bacillary vaccines may make them of some value in combination with other lines of treatment.

Chaulmoogra oil is an old Indian remedy, which one writer thinks was referred to in the ancient writings of Susruta as ' tuvaraka,' but was introduced to western medicine by Mouat in a paper in Vol. I of the *Indian Annals of Medical Science* of 1853-4, and was made official in the Pharmacopoeia of India in 1868 and in the Indian

and Colonial addendum to the British Pharmacopeoia in 1901. There has been a good deal of confusion regarding the origin of the oil, which was for many years erroneously described as being derived from the seeds of *Gynocardia odorata*, until in 1901 Sir David Prain showed that it came from those of *Taraktogenos kurzii* (King) growing along the banks of the rivers of Assam, Chittagong and Burma. It has since been found that various species of *Hydnocarpus*, the most important of which are *H. wightiana* of Southern and Western India, and *H. anthelmintica* of Siam, Indo-China and China, all contain the same active unsaturated fatty acids as *Taraktogenos*, so that it will be convenient to include the oils of both these genera, but not that of *Gynocardia odorata*, under the term chaulmoogra oil.

Much work has been done on the chemical constitution of these oils, Moss, as early as 1879, separating the lower melting point acids under the term gynocardic acid, while Power and Gornall in 1904 and the following years separated first the highest melting point (68° C.) chaulmoogric acid, established its chemical formula and made a number of compounds, including methyl and ethyl chaulmoogrates, and in 1905 Power and Barrowcliff isolated from *H. wightiana* oil, both chaulmoogric and a lower melting point acid (60° C.), named by them hydnocarpic acid, all of which I shall have to refer to again.

Chaulmoogra oil taken orally has long had a reputation in the treatment of leprosy, but has the great disadvantage of being so nauseating that few patients can take sufficient to do more than retard the progress of the disease. The best results have been obtained by Ralph Hopkins, with fifteen years' patient work in the Louisiana Hospital, with 30 per cent. improved, 5 per cent. progressed and 71 per cent. died, of 88 advanced cases; 17 per cent. cured, 4 per cent. lesions disappeared, 48 per cent. improved, 5 per cent. progressed and 12 per cent. died, of 82 incipient cases, showing that only in incipient cases was very material benefit obtained, but demonstrating that the oil had a definite value in leprosy.

A Chinese method of giving the fresh *Taraktogenos kurzii* nuts orally combined with hemp and another Chinese drug has recently been reported by E. A. O. Travers of the Kuala Lumpur Leper Asylum in the Malay States, to have cleared up the symptoms of a certain number of cases of leprosy, while the nuts are very

cheap and readily taken by women and children. He is now, at my suggestion, trying *Hydnocarpus wightiana* nuts, for Reed states that these keep fresh for months if dried, and they can be obtained at about three shillings for 80-lbs. weight, from the Ernakalum Trading Co. of Southern India, being much easier to get than those of *Taraktogenos*. Competent botanists assure me that the *Hydnocarpus wightiana* tree is likely to grow well in any hot, moist climate, with a good rainfall, that is in just those districts where leprosy is so common, and I hope to be able to get the seeds of this species widely distributed before long, if the Malay results are confirmed by further experience.

The importance of the above methods of administration is that they have led to the discovery of more efficient preparations derived from the oils, the evolution of which must now be described.

Gynocardic acid, consisting of the lower melting point fatty acids of the oil, has been used orally since 1891, together with sodium and magnesium gynocardates, with apparent benefit in some cases. I administered gynocardic acid to a few lepers during the first decade of the present century, and came to the conclusion that it was much less irritating to the stomach and more effective than the whole oil, one European patient taking up to forty grains a day for a year, with the result that a very extensive macular leprosy completely cleared up, although some nerve symptoms persisted. As early as 1912 I asked an important firm of manufacturing chemists if they could make for me some soluble compound of gynocardic acid suitable for injection, but unfortunately received a reply in the negative.

In 1911 Engel-Bey reported good results in a few lepers treated orally in Egypt with antileprol, made at his suggestion by separating the free acids of chaulmoogra oil and esterising them, while in 1913 H. Bayon gave this preparation both orally and intramuscularly.

Chaulmoogra oil intramuscularly appears to have first been used successfully by Tourtoules in Egypt, who reported apparent recovery in one case after 650 injections totalling 2,720 grammes in the course of six years, ending in 1899, while Hallopeau in Paris reported benefit in nine lepers treated with combined oral and intramuscular administration, although Castel in 1899 recorded pulmonary embolism in two cases. Jeanselme in 1911 injected

a mixture of chaulmoogra oil, camphor and guiacol, while in 1914 Victor G. Heiser reported from the Philippines 11 per cent. of apparent cures in a small series of cases treated for prolonged periods by intramuscular injections of a mixture of equal parts of chaulmoogra oil, camphorated oil and resorcin, and later Hopkins, McCoy and Hollmann recorded successes by this method, which thus constitutes an important advance.

Sodium Gynocardate Intramuscularly and Intravenously. As the result of Heiser's success with chaulmoogra oil intramuscularly, and of a personal visit from him in Calcutta in 1916, I renewed my attempts to obtain the active portion of the oil in a soluble form suitable for injection, and with the help of Dr. Chuni L. Bose, Professor of Chemistry, and later of the whole time assistance of Dr. Sudhamoy Ghosh, D.Sc., Edin., and with the financial assistance of the Indian Research Association, I obtained first sodium gynocardate, and subsequently similar compounds of the various fatty acids of chaulmoogra, cod-liver and other oils, and investigated their action in numerous cases of leprosy during the next four and a half years. The following results were obtained :—

Intramuscularly sodium gynocardate, prepared from gynocardic acid with a melting point of 37° C., was better borne by Indian patients than the whole oil, and although it produced local pain and induration it gave promising results. I next ascertained by animal experiment that it could safely and practically painlessly be injected intravenously with only very temporary giddiness in a 3 per cent. solution, a large medio-basilic vein allowing of weekly injections for upwards of a year, but when only small veins were available, as is often the case in women and children, irritation of the inner lining of the vein at the immediate site of injection might produce strictly localised and harmless obliterative phlebitis limiting the injections ; this irritation was only partially prevented by the addition of $\frac{1}{2}$ per cent. sodium citrate to the solution. I next found that the sodium salts of chaulmoogra oil, fatty acids with melting points of from 49° to 51° C., containing both gynocardic and hydnocarpic acids, and which I called gynocardate of soda A, were more effective in leprosy than those of the lower melting point acids, while the salts of chaulmoogric acid itself were much less soluble and also less active. Eventually I came to the conclusion

that the salts of the whole of the fatty acids of *Hydnocarpus wightiana* oil, containing more hydnocarpic and less chaulmoogric acid that the oil of *Taraktogenos kurzii*, gave the best results, and this oil has since been used in Calcutta by Muir and by many other workers in the East in making preparations for injections in leprosy, the tedious and expensive process of fractionation being unnecessary now that these points have been established by careful investigations. Professor B. E. Reed of the Peking Union Medical College, has recently stated that *Hydnocarpus* preparations, mainly supplied from Calcutta, have found favour in Singapore, the Malay States, Burma and elsewhere, and he concluded that ' the antiquity of the records of *hydnocarpus*, the continuity of its use in many countries, its high chemical and therapeutic worth, give it a place of international importance.'

Reactions with Destruction of the Lepra Bacilli due to Gynocardates and Hydnocarpates. The subcutaneous injections of sodium gynocardate produced gradual improvement in leprosy cases, without the occurrence of any marked reactions in the affected tissues, but when I commenced to give the preparation intravenously a remarkable and hopeful phenomenon was observed, nearly always in rather advanced nodular cases with enormous numbers of lepra bacilli in the affected tissues. The reaction which took place is well illustrated by a coloured plate I published in 1919 showing inflammatory swelling and softening of the leprosy infiltrated lobes of both ears, while microscopical examinations of excised portions in such cases revealed only a few remaining typical rod-shaped acid-fast bacilli, together with innumerable acid-fast granules of disintegrated organisms, demonstrating that the inflammatory local reactions produced by this vegetable substance had resulted in the destruction within the human tissues of enormous numbers of the pathogenic organism, and opening up possibilities of an important advance in the treatment of this hitherto intractable disease. Fever for a day or two always accompanies such reactions, which may be induced by very minute doses of the drug, and very occasionally fever may persist for one or more months. This is accompanied by a softening of numerous nodules and a considerable degree of toxaemia resulting in prolonged debility, while a number of new skin lesions may appear in the form of slightly

raised red patches, just as occurs naturally, from time to time, in the more acute cases of nodular leprosy in what Muir calls the reactionary phase of untreated cases. As few bacilli are found in reaction lesions they may largely be due to inflammatory reactions excited in small deposits of lepra bacilli, which had not previously produced visible lesions while still quiescent. As a rule such reactionary lesions appearing during treatment clear up again rapidly, although occasionally some of them persist and the patient appears to be worse for a time, so it is now generally considered advisable to try as far as possible to avoid the more severe reactions by cautious dosage in the active second stage of the disease. Muir, however, finds that in the first stage of very limited lesions, as well as in the third quiescent stage, when the reactionary phase no longer occurs naturally owing to the establishment of tissue resistance to the toxins of the bacillus, the treatment may safely be pushed with beneficial effects. Considerable experience, both as regards the natural course of the different varieties of leprosy and also in the exhibition of the powerful remedies now available, is thus necessary to enable the best results to be obtained, which is, doubtless, the reason why some observers have failed to get good effects in their earlier attempts to use the new treatment.

Severe febrile and local reactions are, however, exceptional, steady improvement in their absence far more frequently ensuing, although careful observations in the wards of leper institutions enabled Muir to observe that slight rises of temperature, not noticed by the patient, nearly always occur in patients showing fairly extensive bacteriologically positive lesions, indicating the destruction of smaller numbers of bacilli of a beneficial nature. I was also able to demonstrate by repeated microscopical examination of small excised portions of nodules from the ear or other affected part, that in the entire absence of any reactions noticeable to the patient a gradual breaking up and diminution of the lepra bacilii was brought about by repeated injections of these preparations, accompanied by slow absorption of the nodules, until nothing but a few acid-fast granules could be detected; this stage, in turn, was soon followed by the entire disappearance of the bacilli from the tissues, as well as from the nasal mucus, rendering the patients apparently free from both all the symptoms and infectivity of the disease,

six to eighteen months usually being required to bring about this happy result in typical, but not extremely advanced, nodular cases.

In anaesthetic cases with nodular thickening of the ulnar nerves, I have also seen reactions consisting of temporary swelling of the nodules accompanied by severe pain, sometimes necessitating the use of morphine, but followed by subsidence and eventual great improvement of sensation in the area supplied by the affected nerve, while in less advanced cases steady, but usually slow, return of both sensation and muscular power occurred, together with eventual disappearance of the depigmented patches in various parts of the body. One of the earliest cases of this type, with foot drop greatly crippling him, lost all visible signs of the disease and became able to walk ten miles at a stretch. On the other hand, when nerve trunks of the extremities have been extensively destroyed by prolonged disease, and fingers and toes have been lost, or the typical claw hand with wasting of nearly all the intrinsic muscles has developed, complete restoration of function is obviously impossible, although a considerable degree of recovery of sensation and muscular power may take place when the disabilities are of recent origin, but not in the long standing crippled cases, so frequently seen in Indian leper asylums, with permanent destruction of extensive portions of their distal nerve trunks by irremovable fibrous scar tissue. Such wrecks of humanity may remain in a stationary condition for several decades before some intercurrent disease releases them from their misery, and the disease can only be prevented from reaching this incurable stage by effective treatment at an early period.

An even more remarkable and important phenomenon which I have observed in a very few bad nodular cases, who developed most prolonged and severe febrile reactions after even a single minute dose of sodium gynocardate, with great debility and inflammatory softening of extensive lesions, has been a steady improvement extending over many months, without any further treatment, and even complete recovery during the ensuing year; of this type of case the following are examples: One of the worst cases I have seen of nodular leprosy of twenty years' duration, with extreme thickening of the skin of the face and extensive ulceration of both the ears and

the hands, had three months fever after the minute intravenous dose of 0·2 c.c. of a 3 per cent. solution of sodium gynocardate, followed by steady improvement without any further treatment, and at the end of a year only loose folds of skin remained at the site of the former facial nodules, and the ears and hands had completely healed. Another patient with a ' grog blossom-like ' nose and raised red leprotic patches the size of the palms of the hands on his body, after two months reactionary fever following a few small doses of the same drug with great loss of strength, was given no further treatment except sodium morrhuate orally, although he was anxious to continue the injections. I sent him away for a change as soon as he began to pick up, and saw him again about a year after the reaction, when only slight pitting with some fibrous scarring remained at the sites of the former extensive lesions, sections of removed portions being quite free from acid-fast bacilli; complete recovery had thus taken place. Such cases are quite exceptional, but their occurrence at least indicates that the action of chaulmoogra oil soluble preparations cannot be explained solely by any direct destructive effect on the lepra bacilli: a point of great theoretical importance which I shall return to in the last lecture.

Sodium Morrhuate and Sodium Soyate in Leprosy. The destruction of the lepra bacillus after intravenous injections of gynocardates naturally led me to consider the possibility of inducing a similar change in the acid-fast bacillus of tuberculosis, and I consequently got Dr. S. Ghosh to extract the fatty acids from cod-liver oil and make a sodium salt for me, which I called sodium morrhuate. I found it made a clear solution almost unirritating both by subcutaneous and intravenous injection, so I next tried it by both methods in leprosy, and soon observed that it could induce by either mode of administration febrile reactions in leprotic tissues with destruction of the lepra bacillus, followed by similar improvement and ultimate disappearance of all signs of the disease. This will be evident from the coloured drawing I show you, in which six months' treatment with practically painless subcutaneous injections of a 3 per cent. solution of sodium morrhuate brought about the absorption of very numerous raised circinate red patches on all parts of the body of the patient, with disappearance of the lepra bacilli, leaving only lighter depigmented patches, although it should be

mentioned that he left off treatment against advice at this stage and I found a slight recurrence in one spot a year later.

As the value of chaulmoogra oil had previously been attributed to its possessing a closed carbon ring, a unique constitution for a fatty acid, the activity of sodium morrhuate in leprosy disproved that interesting theory, and led me to think that the proportion of unsaturated fatty acids as a class might be the most important factor influencing the therapeutic value of oils in leprosy. To test this theory I next selected some oils with a high iodine value, including soya bean oil and Japanese sardine oil, and had similar sodium salts of their unsaturated fatty acids prepared ; I found the latter to be irritating to human tissues, but the former, which I called sodium soyate, formed a clear and unirritating 3 per cent. solution suitable for injection either subcutaneously or intravenously. There was only time to try it in a few cases before I left India, in one of which an extensive, red, raised leprotic patch covering the whole of one cheek, together with smaller ones on other parts of the body, completely disappeared and the tissues became bacteriologically negative in the remarkably short period of six weeks ; this is by far the most rapid improvement I ever saw in a case of that degree, although the effects were much less rapid in the other cases, and as far as I know, this preparation has not since been tried on a sufficient scale to decide its precise value. The few tests I did, add yet another oil to those furnishing active preparations against the acid-fast bacillus of leprosy. In 1919, Dr. K. K. Chaterji, of Calcutta, applied my methods to nim oil, and reported great benefit in two lepers treated by ' margosates ' thus prepared. During the last two years, Muir has obtained active preparations on the same lines from linseed and olive oils, although he concluded that those prepared from the ' closed ring ' fatty acids of chaulmoogra oil were rather more effective in leprosy than the others, while preparations from saturated fatty acids had very little effect in this disease. This largely confirms my theory, and at the same time opens up an unlimited field of research on the many oils, which may furnish still more effective preparations, both in leprosy, and possibly also in tuberculosis.

Results of Treatment with Gynocardates and Hydnocarpates and with Sodium Morrhuate. In 1917, I reported twenty-six leprosy cases

treated for three or more months with sodium gynocardates and hydnocarpates with improvement in all, while eight of the twelve treated for a year or more had lost all signs of the disease, and added 'whether permanent results can be obtained only time will reveal.' In 1919, I recorded fourteen cases treated with sodium morrhuate, and the following table shows the results obtained in all the cases I had treated for three months and over, when I left India early in 1920, the sodium morrhuate series being shown separately.

TABLE III

	Not Improved	Slightly Improved	Greatly Improved	Lesions all disappeared	Total Cases
Gynocardates and Hydnocarpates— 3 months and over	1	9	20	21	51
Gynocardates and Hydnocarpates— over 1 year	1	1	2	9	13
Sodium morrhuate— 3 to 12 months	0	3	12	5	20

Thus, with the chaulmoogra oil preparations, in round numbers, 40 per cent. had cleared up completely and another 40 per cent. had so greatly improved that there was very good probability of their losing all signs of the disease with further treatment, giving 80 per cent. of good results, while of 13 cases treated for the sufficient period of a year or more, 9, or 69 per cent., had cleared up completely. The sodium morrhuate cases had been treated for shorter periods of from three months to one year and of 20 cases 12 had greatly improved and 5 had completely cleared up, the results being about equal to those with the gynocardates and hydnocarpates when the duration of treatment is taken into account. In only one very advanced nodular case of the total 71 cases had no improvement resulted, but it must be mentioned that as the cases represented all stages of leprosy, including some early ones, they were considerably more favourable than the average type met with in leper asylums.

As I pointed out in 1919, relapses occur in some cases which leave off the treatment as soon as the lesions have disappeared, and of 34 cases followed up since my report of 1917, one remained unimproved, 10 had further improved under continued treatment, in 5 the lesions

had now disappeared, 13 remained clear of symptoms and 5 had relapsed—the latter, all cases in Indians who had left off the treatment prematurely against my advice. These results clearly indicate the necessity of continuing injections for some months, or better still a year, after the disappearance of outward signs of the disease, as was only to be expected in a chronic affection due to a highly-resistant organism. Some of the relapsing cases cleared up again on resuming treatment. Further cases becoming stationary on hydnocarpate treatment, may proceed to clear up completely on using sodium morrhuate, illustrating the advantage of having more than one effective remedy.

Disadvantages of the Intravenous Method. The necessity of giving sodium hydnocarpate intravenously to obtain the best results is a serious disadvantage, on account of its irritant action on the veins, leading to obliteration, and the time consumed as compared with an intramuscular injection. Further, sodium morrhuate has been found to deteriorate in solution through oxidation, while it is troublesome and expensive to put it up in capsules to avoid change. Dr. Ghosh made some ethyl hydnocarpate for me, which I had not the time to try to any extent before I left India, and the next advance is due to American workers.

Ethyl Ester Chaulmoogrates Intramuscularly. In 1919 Dr. H. T. Hollmann and Professor Dean of Honolulu reported on the use of intramuscular injections of ethyl esters of the different fractions of the fatty acids of chaulmoogra oil, and confirmed my conclusion that the chaulmoogric acid fraction produced little effect, but that the lower melting point ones were more active. They obtained marked improvement in 17 out of 26 cases, improvement in 3, slight improvement in one and no improvement in 3, who had been treated for three months or less, very similar results to my earlier Calcutta ones. Their method had the great practical advantage that the ethyl ester could be injected in a pure fluid state into the gluteal muscles without much pain, enabling a large number of cases to be dealt with in a comparatively short time. This convenient modification has since been very generally adopted in many parts of the world with slight variations, Muir having introduced a very convenient formula, which in its latest form consists of equal parts of ethyl hydnocarpate (prepared from *H. wightiana*) and of pure olive oil,

with 4 per cent. double distilled creosote (E.C.O. mixture), his former E.C.C.O. mixture having also contained camphor (which J. G. Samson and G. Limkako at Culion found to be useless, although they confirmed the value of the addition of creosote), or 10 per cent. thymol (E.T.O.) may be substituted for the creosote. Muir advises injections of these mixtures either beneath the leprous lesions or intragluteally, or both, in doses rising by 0·5 c.c. at a time from 0·5 c.c. up to a maximum of 10 c.c. once or twice a week, the next dose being reduced if the temperature rises to 100° F. and remains up for more than 24 hours, while if any marked reaction occurs in the diseased tissue, injections should be stopped until it has completely subsided.

At my suggestion Muir tried giving iodide of potassium orally in daily doses of from ½ to 20 grains, and found this addition may induce reactions after ethyl hydnocarpate when absent without it, resulting in further improvement, and he has also used a vaccine of Kedrowsky's acid-fast bacillus with success for the same purpose : illustrating the principle that a variety of substances, that I showed in the historical review of former treatments have produced reactions in leprosy, may be of value to supplement the action of the new methods. Time does not permit me to go into further detail regarding the treatment of different stages of leprosy, which will be dealt with in a work on which Dr. Muir and I have been long engaged, and I must pass on to consider the results which have been obtained in other countries where the different remedies have been tested, the most extensive trials of which we owe to American workers.

In India I arranged, through the kindness of the Mission to lepers, for a trial of both sodium gynocardate and sodium morrhuate separately in thirteen leper asylums, and asked Dr. Muir to make an independent analysis of the results. 300 cases showed 72 per cent. improved and 32 per cent. greatly improved although the treatment had only lasted for from two to twelve months, and those treated for six months and over to a year gave 100 per cent. improved and 52 per cent. greatly improved, the more promising cases having been selected for treatment, while the two preparations gave almost identically the same results, establishing the principle that those from other oils than chaulmoogra may be effective in leprosy.

Results with Ethyl Chaulmoogrates and Hydnocarpates. It is still too early to allow of anything like final conclusions regarding the precise value of the new treatment in leprosy, but the following extensive trials will afford some indications. During the last two years a great test was begun in the Culion settlement under Dr. H. W. Wade, who has kindly sent me a preliminary report showing that treatment was begun first in May, 1921, 500 cases being treated during the next two months, and gradually increased to 1,500 by April, 1922, and 4,067 in the following year. A survey in September, 1923, showed improvement in 55·9 per cent., while in 36 per cent. more the progress of the disease had been checked, 6·4 per cent. were worse, and 1·7 per cent. had died, and concluded that under the circumstances 'it is felt that this result is far from discouraging.' This opinion is strengthened by the following figures which speak for themselves, showing the percentages improved after different periods of treatment.

Months of Treatment ...	Under 3	3–6	6–9	9–12	12–15
Percentage Improved ...	26 %	42 %	74 %	81 %	93 %

In Hawaii still more instructive figures are now available on account of the much longer duration of the treatment by ethyl chaulmoogrates at the Kahili hospital near Honolulu, where all newly discovered lepers are first sent for diagnosis and treatment, and the most advanced unamenable ones eventually drafted on to the Molokai settlement. From 1912 to 1918, before the introduction of the new improved treatment, the yearly discharges on parole as recovered averaged 6·5, during the first two fiscal years ending on June 30th, 1919, and 1920, of the new treatment, 20 and 31, respectively, were thus discharged, in 1920-21 there were 115 new admissions and no less than 94 discharged recovered; of these 23 relapsed, so in 1921-22 under stricter rules only 26 were paroled, the number rising again the following year, 1922-23, to 52. During the last three years, when the full effects of the improved treatment were obtained, there were 310 admissions and 172 discharged recovered, or 55·5 per cent. of the number admitted during the same period, all after examination by a board of three experienced doctors; 16 died of complicating diseases, mostly from tuberculosis. During the last four years 92 cases were transferred to Molokai as not yielding to treatment, a yearly average of 22·3 per cent., or less than one-fourth of the average yearly admissions. Further, of a total

of 249 paroles in the ten years, 1912-21, 31, or 12·4 per cent. relapsed, some of whom cleared up again on further treatment.

To understand the full significance of these figures we must recall that the usual annual mortality among the advanced cases seen in leper asylums and settlements is rarely less than 10 per cent., and is often higher ; the recent Hawaii admissions of the last three years show a diminution each year of about 10 per cent. on the numbers of the previous year, which it is not unreasonable to hope may continue, in view of the numbers of cases now being cleared up in the early stages before they have had the prolonged opportunities of infecting their households, as when they hid their disease in the absence of any efficient treatment, instead of declaring it voluntarily as so many have done recently. If such proves to be the case I estimate that the total known Hawaii lepers will decline by over 40 per cent. within one decade, or far more rapidly than has ever been known in a tropical country in the absence of any effective treatment : clearly illustrating the value of the new methods in the age-long struggle to stamp out leprosy.

The immense importance of treating leprosy in its early stages is proved by the following Honolulu figures of the percentages of recovered cases in relation to the duration of the disease on commencing treatment.

Years Duration...	Under ½	½–1	1–2	2–3	3–4	4–5	5–8	8–10	Over 10
Recovered ...	44%	18·5%	17%	10·5%	9%	4%	4%	3%	9·5%

The much higher recovery rate in early cases is well shown by these figures, while in my own cases I found that 50 per cent. of cases treated within three years of the onset cleared up completely, but only 25 per cent. of those from three to fifteen years' duration did so. It is also of great significance that in Hawaii in former days the discharge as recovered scarcely ever occurred in the nodular type, while under the new treatment just over two-thirds of those released on parole were of this previously incurable form. The younger age groups also yielded the largest proportion of successes.

These results are also in accordance with Muir's recent statement : 'We have found, further, that most early cases lose all signs of active disease within a few months. . . . The most hopeful method of dealing with leprosy must, therefore, depend on early diagnosis and treatment.' Dr. Travers, who is now in charge of a leper asylum

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In Hawaii still more instructive figures are now available on account of the much longer duration of the treatment by ethyl chaulmoogrates at the Kahili hospital near Honolulu, where all newly discovered lepers are first sent for diagnosis and treatment, and the most advanced unamenable ones eventually drafted on to the Molokai settlement. From 1912 to 1918, before the introduction of the new improved treatment, the yearly discharges on parole as recovered averaged 6·5, during the first two fiscal years ending on June 30th, 1919, and 1920, of the new treatment, 20 and 31, respectively, were thus discharged, in 1920-21 there were 115 new admissions and no less than 94 discharged recovered; of these 23 relapsed, so in 1921-22 under stricter rules only 26 were paroled, the number rising again the following year, 1922-23, to 52. During the last three years, when the full effects of the improved treatment were obtained, there were 310 admissions and 172 discharged recovered, or 55·5 per cent. of the number admitted during the same period, all after examination by a board of three experienced doctors; 16 died of complicating diseases, mostly from tuberculosis. During the last four years 92 cases were transferred to Molokai as not yielding to treatment, a yearly average of 22·3 per cent., or less than one-fourth of the average yearly admissions. Further, of a total

of 249 paroles in the ten years, 1912-21, 31, or 12·4 per cent. relapsed, some of whom cleared up again on further treatment.

To understand the full significance of these figures we must recall that the usual annual mortality among the advanced cases seen in leper asylums and settlements is rarely less than 10 per cent., and is often higher ; the recent Hawaii admissions of the last three years show a diminution each year of about 10 per cent. on the numbers of the previous year, which it is not unreasonable to hope may continue, in view of the numbers of cases now being cleared up in the early stages before they have had the prolonged opportunities of infecting their households, as when they hid their disease in the absence of any efficient treatment, instead of declaring it voluntarily as so many have done recently. If such proves to be the case I estimate that the total known Hawaii lepers will decline by over 40 per cent. within one decade, or far more rapidly than has ever been known in a tropical country in the absence of any effective treatment : clearly illustrating the value of the new methods in the age-long struggle to stamp out leprosy.

The immense importance of treating leprosy in its early stages is proved by the following Honolulu figures of the percentages of recovered cases in relation to the duration of the disease on commencing treatment.

Years Duration...	Under ½	½–1	1–2	2–3	3–4	4–5	5–8	8–10	Over 10
Recovered ...	44%	18·5%	17%	10·5%	9%	4%	4%	3%	9·5%

The much higher recovery rate in early cases is well shown by these figures, while in my own cases I found that 50 per cent. of cases treated within three years of the onset cleared up completely, but only 25 per cent. of those from three to fifteen years' duration did so. It is also of great significance that in Hawaii in former days the discharge as recovered scarcely ever occurred in the nodular type, while under the new treatment just over two-thirds of those released on parole were of this previously incurable form. The younger age groups also yielded the largest proportion of successes.

These results are also in accordance with Muir's recent statement : 'We have found, further, that most early cases lose all signs of active disease within a few months. . . . The most hopeful method of dealing with leprosy must, therefore, depend on early diagnosis and treatment.' Dr. Travers, who is now in charge of a leper asylum

in the Malay States after long service there, has recorded his opinion that ' if taken in time, the progress of the disease can be arrested and that in a large proportion of cases, leprosy can be actually cured.' Muir also states ' Our experience shows that leprosy can almost always be diagnosed long before it becomes infectious ; that is to say, the disease may be recognised by clinical signs long before bacteriological examinations are positive,' and he rightly, in my opinion, advocates the multiplication of leprosy clinics, something on the lines of tuberculosis dispensaries in Britain, but with far brighter prospects of successfully eradicating the plague.

Can Lasting Cures be Obtained ? It is now established that most early and some more advanced cases lose all symptoms and infectivity of leprosy under treatment, but it is still too early to say in what proportion of such cases the whole of the lepra bacilli have been destroyed with consequent permanent cure apart from reinfections. The frequency with which relapses occur in tuberculosis after apparent cure by sanatorium treatment necessitates the greatest caution in claiming permanent results in leprosy before sufficient time has elapsed to justify these claims. Nevertheless, the outlook even in this respect appears to me to be far more favourable than in tuberculosis, for one of my early patients has now remained free for over eight years from all signs of the disease, except for the crippling of one hand due to irreparable damage to an ulnar nerve, although he has had no treatment during the last three years ; several more cases had remained free from all symptoms for five and six years when I last heard from them. In Hawaii 88 per cent. of the paroled cases have remained well for several years, and although it is advisable to continue some treatment for a year or so after apparent recovery, yet with this precaution there are now good grounds for hoping that the results will be permanent in a large proportion of the earlier cases at any rate. Moreover, the evidence that the treatment actually leads to destruction of the lepra bacillus in the body not only places it in a more hopeful position than the building up of the resisting powers of the tissues by the sanatorium treatment of tuberculosis, but also raises the still more important question of the possibility of applying the new line of treatment to the white man's scourge, tuberculosis.

RAVAGES CAUSÉS PAR LA MOUCHE DE GOLOUBATZ EN ROUMANIE ; SES ATTAQUES CONTRE LES ANIMAUX ET CONTRE L'HOMME

PAR

T. CIUREA

PROFESSEUR DE PARASITOLOGIE À LA FACULTÉ DE MÉDECINE VÉTÉRINAIRE DE BUCAREST

ET

DR. G. DINULESCU,

VÉTÉRINAIRE CAPITAINE DE L'ARMÉE ROUMAINE

PLATES XVIII-XX

I. LES ATTAQUES CONTRE LES ANIMAUX

INTRODUCTION. En Europe, un des plus grands foyers de Simulies est celui représenté par la mouche de Goloubatz (*Simulium columbaczense* Schiner)*, dont l'origine se trouve en Jugoslavie dans les départements de Pojarevatz et Craina du côté droit du Danube et en Roumanie dans les départements du Banat : Timis-Torontal et Caras-Severin du côté gauche. Dans cette région montagneuse, qui mesure environ 22000 Km. il n'existe guère de petits cours d'eau, où l'on ne puisse trouver l'une des formes évolutives de cette Simulie (Tömösváry).

De ce foyer à peu près chaque année au printemps,† la mouche de Goloubatz fait ses invasions en essaims plus ou moins grands dans quelques-uns de nos départements de Transylvanie et d'Olténie. La population rurale de ces départements connait assez bien la mouche et plus ou moins aussi les moyens pratiques d'en protéger le

* *Simulium columbaczense* a pris son nom de l'ancien château de Goloubatz situé sur la rive droite du Danube, dans le département de Pojarevatz en Jugoslavie ; on croyait autrefois, que cette Simulie faisait son évolution principalement dans les petits ruisseaux, qui coulent dans le voisinage de ce château.

D'après les recherches que nous avons entreprises sur les Simulies de la faune de Roumanie, il s'agit ici, non seulement du *Simulium columbaczense*, mais aussi d'autres espèces de *Simulium*, dont les nymphes possédant 4, 6 et 8 trachées de chaque côté, se trouvent en grand nombre dans les petits ruisseaux de cette région. Nous espérons pouvoir revenir sur cette question lorsque notre étude sera terminée.

† Dans le Banat d'après une légende locale, les mouches de Goloubatz s'envolent au printemps d'une grotte nommée „ Gaura cu musca " (la grotte à la mouche) qui se trouve sur la rive gauche du Danube, dans les montagnes du département de Caras-Severin, près du village de Coronini. D'après cette légende les mouches prennent naissance ici de la tête putréfiée d'un dragon tué par un Hercule.

bétail, de sorte que le nombre d'animaux tués annuellement par cette Simulie, ne dépasse que rarement quelques dizaines de victimes. Mais exceptionnellement cette année (1923), d'immenses essaims de Simulies ayant l'apparence de nuages, ont invadé non seulement le Banat, la Transylvanie et l'Olténie (à l'exception du département de Romanati), mais aussi une grande partie de la Valachie, où la population ne connaissait pas la mouche de Goloubatz et les moyens d'en protéger les animaux. C'est ainsi qu'on peut expliquer qu'en quelques jours plus de 16000 animaux ont été tués par les piqûres vénimeuses de cet insecte. La mouche a tué aussi de nombreux animaux sauvages tels que chevreuils, lièvres et renards. Les hommes même eurent à en souffrir.

Les deux essaims de Simulies ; le temps de leur apparition ; la direction qu'ils ont suivie ; les départements qu'ils ont invadé et la durée de leur invasion. D'après les informations fournies par les Médecins Vétérinaires, les mouches de Goloubatz ont fait leur apparition en petit nombre dans le département de Caras-Severin du Banat. Vers le milieu du même mois elles sont devenues plus nombreuses et ont formé deux grands essaims, dont l'un s'est dirigé tout d'abord vers le Nord et l'autre un peu plus tard vers l'Est.

(*a*) *Le premier essaim*, dirigé vers le Nord, a invadé les départements suivants du Sud-Ouest du Banat et de la Transylvanie :

Le 15 Avril les départements de Timis-Torontal et de Hunedoara, le 26 Avril le département de l'Arad, le 3 Mai le département de Bihor, le 8 Mai le département d'Alba de jos et le 10 Mai le département de Turda-Aries. La progression des mouches s'est arrêtée ici.

Dans le Nord, l'invasion de cette région dura environ jusqu'aux derniers jours du mois de Mai. Dans le Sud, spécialement dans le département de Caras-Severin, elle a été de plus longue durée, —pendant tout le mois de Juin. Les autorités de la circonscription de Moldova-Nouă ont rapporté que l'invasion s'est prolongée ici jusqu'à la fin du mois de Juillet, mais la commission chargée de l'étude de ces insectes n'a pas pu les observer même dans les premiers jours de ce mois.

(*b*) *Le second essaim* de mouches de Goloubatz, en partant aussi du département de Caras-Severin ou des régions voisines s'est dirigé vers l'Est, un peu plus tard que celui du Nord et a invadé d'abord huit départements de l'Olténie et de la Valachie (spécialement

ceux qui sont situés le long du versant méridional des Carpathes) dans l'ordre suivant :

Le 21 Avril le département de Mehedinti, le 23 Avril les départements de Doljet de Gorj, le 25 Avril le département de Vâlcea. Le 28 Avril elles sont passées en Valachie dans les départements de l'Olt et l'Arges, le 30 Avril dans le département de Muscel et le 2 Mai dans le Nord du département de Dâmbovita. L'invasion fut arrêtée ici par une pluie, qui a fait disparaître les mouches.

Ensuite par les défilés des Carpathes de l'Olténie et de la Valachie, les mouches de Goloubatz ont invadé trois départements de la Transylvanie situés sur le versant septentrional de ces montagnes. Le 23 Avril elles sont passées du département de Gorj dans celui de l'Hunedoara ; le 4 Mai les mouches du département de Vâlcea ont invadé le département de Sibiu et le 5 Mai les Simulies de Muscel ont passées dans le département de Fagaras.

L'invasion a duré dans ces départements jusqu'aux derniers jours du mois de Mai, c'est à dire plus de 40 jours.

En résumé on peut dire que ces Simulies ont invadé 17 départements de la Roumanie, dont 2 appartiennent au Banat, 7 à la Transylvanie, 4 à l'Olténie et 4 à la Valachie, ce qui représente environ un huitième de la surface totale du pays.*

Les mouches de Goloubatz ont été apporté par le vent. Il y a deux opinions en ce qui concerne le mouvement des essaims de Simulies : d'après l'une, les essaims se déplacent en remontant le long des grands cours d'eaux ; d'après l'autre, ils sont transportés passivement par le vent. Si nous observons sur la charte d'invasion ci-joint la direction suivie par les Simulies, nous voyons qu'elle est perpendiculaire sur celle des cours d'eaux. Ce fait démontre qu'il s'agit ici d'un transport passif de ces insectes. D'ailleurs à l'occasion de l'enquête que nous avons faite parmi les Médecins Vétérinaires relativement aux différentes questions se rapportant à l'invasion,

* En même temps que la Roumanie, la Jugoslavie et la Bulgarie furent envahies par les Simulies, mais le nombre d'animaux tués dans ces deux derniers pays fut beaucoup moins que celui de la Roumanie.

D'après les rapports officiels de la Jugoslavie, la mouche a invadé sept départements du Sud-Est du pays (Vrania, Morava, Pojarevatz, Pirot, Krusevatz, Timoc et Toplic), où elle a tué 1552 animaux, dont 25 chevaux, 1 âne, 910 bovins, 251 moutons, 90 chèvres et 295 porcs.

En Bulgarie furent attaqués trois départements du Nord-Ouest (Widin, Vrata et Sophia). Dans le premier département la mouche de Goloubatz a tué 1500 animaux, dont 42 chevaux, 4 ânes, 503 bovins, 264 buffles, 306 moutons, 183 chèvres et 199 porcs. Dans les deux autres départements aussi la mouche a tué un grand nombre d'animaux.

TABLEAU I

Animaux tués par la mouche de Goloubatz pendant l'invasion de l'année 1923.

Provinces	No. courants	Départements	No. des communes invadées	Chevaux	Ânes	Bovins	Buffles	Moutons	Chèvres	Porcs	Total par départements	Total par provinces
BANAT	1	Caras-Severin ...	42	5	1	42	—	—	2	4	54	1500
	2	Timis-Torontal ...	20	210	—	1221	15	—	—	—	1446	
TRANSYLVANIE	3	Hunedoara	189	22	1	269	—	9	20	19	340	659
	4	Arad	35	2	—	77	—	9	37	11	136	
	5	Bihor	26	1	—	53	—	1	10	5	70	
	6	Alba de Jos	10	1	—	41	—	—	—	—	42	
	7	Turda-Aries	9	—	—	5	—	—	—	—	5	
	8	Sibiu	13	5	—	57	—	—	—	2	64	
	9	Fagaras	2	—	—	2	—	—	—	—	2	
OLTÉNIE ...	10	Mehedinti	145	216	—	1055	—	106	13	102	1492	8178
	11	Dolj	82	312	20	1249	—	60	—	175	1816	
	12	Gorj	114	107	11	560	—	281	84	520	1563	
	13	Vâlcea	111	391	10	1756	12	114	120	940	3307	
VALACHIE ...	14	Arges	75	184	6	2884	—	298	113	952	4437	6137
	15	Muscel	54	121	11	1027	1	37	24	117	1338	
	16	Olt	9	4	—	106	—	—	—	12	122	
	17	Dâmbovita	9	4	—	188	—	—	37	11	240	
		Total général ...	945	1585	60	10,592	28	915	460	2,834	16,474	

presque tous nous ont fait savoir que les mouches ont été apportées de la direction de leurs lieux d'origine par le vent, qui a soufflé quelque temps avant l'invasion.

En dehors de ce mode de transport des Simulies à de grandes distances, nous croyons, comme d'autres auteurs, que ces insectes peuvent se déplacer à des distances moins longues, d'une localité à l'autre en accompagnant les animaux qu'ils désirent attaquer. Ainsi on a vu fréquemment les villages envahis le soir par des Simulies venant avec les animaux qui rentraient des pâturages.

Le nombre d'animaux tués, leur valeur marchande et l'indemnisation accordée par le gouvernement roumain aux fermiers les plus éprouvés.

D'après les dates, qui nous ont été fournies par le Ministère de l'Agriculture, les mouches de Goloubatz ont tué pendant l'invasion 16,474 animaux domestiques, dont la répartition par espèces, provinces et départements se trouve dans le Tableau I ci-joint.

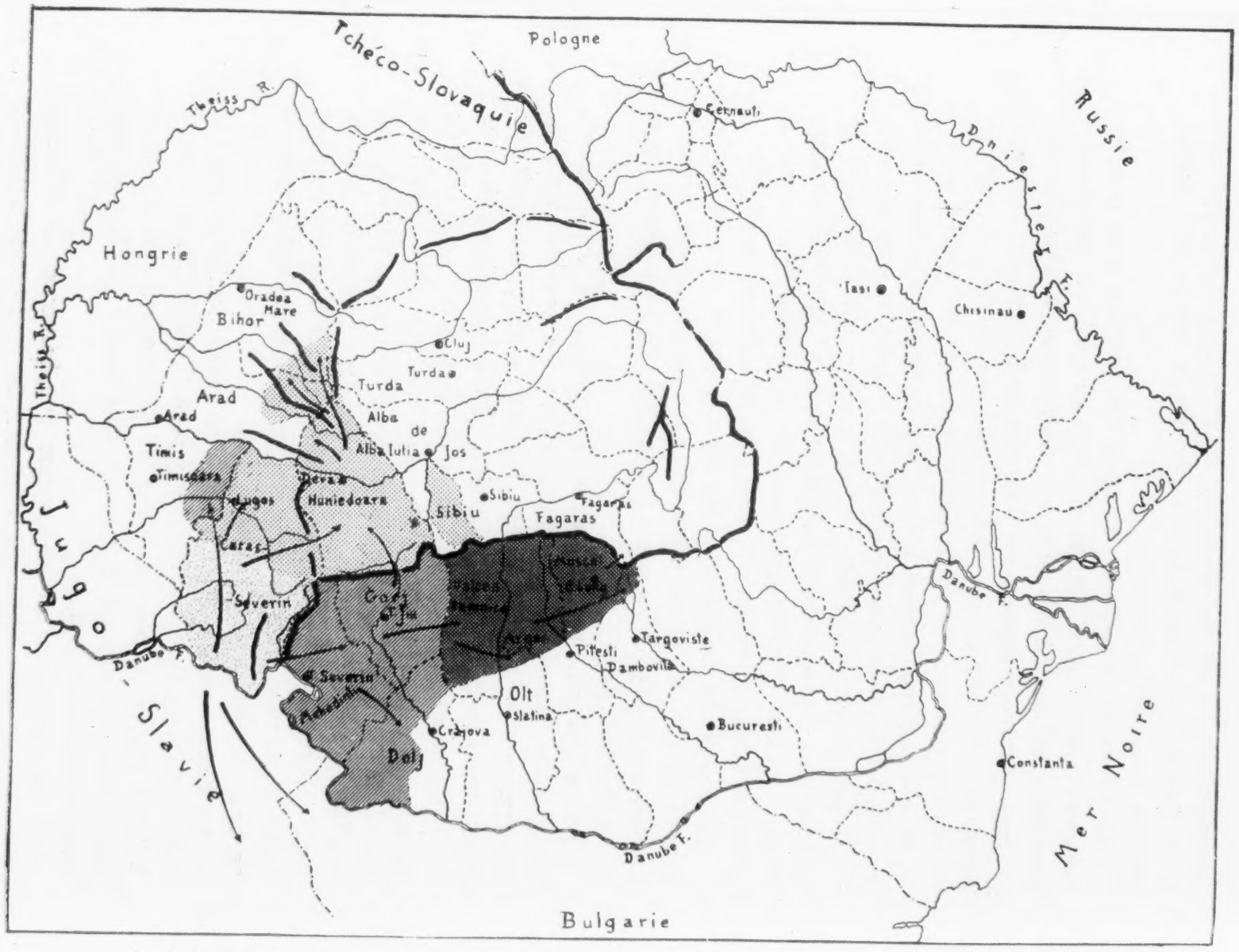
En consultant ce tableau nous voyons, que ce sont les bovins qui ont payé le plus grand tribut à la mort (10,592), après viennent les chevaux (1,585) et les porcs (2,834), ensuite les moutons (915) et les chèvres (460).

En ce qui concerne la gravité des pertes, les départements peuvent être rangés dans l'ordre suivant : En première ligne les départements de l'Arges, de Vâlcea, de Muscel et une petite portion du département de l'Olt et de Dâmbovita. En deuxième ligne viennent les départements de Mehedinti, de Dolj, de Gorj et une partie du département de Timis-Torontal. En troisième ligne sont : la moitié du Nord du département de Caras-Severin, le département de l'Hunedoara et les parties invadées des départements de Alba de Jos, de Turda-Aries, de l'Arad, de Bihor, de Sibiu et de Fagaras. Enfin en quatrième ligne vient la moitié méridionale du département de Caras-Severin (voir la charte).

En général on peut dire que le plus grand nombre d'animaux tués s'est trouvé dans les départements de l'Olténie (8,178) et de la Valachie (6,137), tandis que dans les départements du Banat ce nombre a été seulement de 1,500 et dans ceux de la Transylvanie encore plus réduit (659).

Il est intéressant de mentionner que beaucoup de Médecins Vétérinaires, ont rapporté que les piqûres des mouches de Goloubatz ont été mortelles seulement pendant les premiers quatre ou cinq

CHARTE DE LA ROUMANIE ; LES DÉPARTEMENTS INVADÉS PAR LA MOUCHE DE GOLOUBATZ
AU PRINTEMPS DE L'ANNÉE 1923.



jours de l'invasion, quoiqu'elles soient restées plus de deux ou trois semaines dans une même localité et que leurs piqûres fussent aussi nombreuses qu'au commencement de l'invasion. Cela s'explique, nous croyons, par l'établissement d'un état d'immunité chez les animaux qui ont resisté à une première attaque. Cette hypothèse est corroborée d'ailleurs par le fait que la mortalité la plus réduite a été constatée parmi les animaux de la moitié inférieure du département de Caras-Severin, c'est à dire là, où la mouche sévit d'une manière endémique. À cela s'ajoute certainement le fait que la population de cet endroit est mieux renseignée sur les moyens de prévention contre l'attaque de l'insecte.

Dans le Tableau II ci-joint nous avons calculé le pourcentage des pertes dans les départements les plus ravagés seulement.

TABLEAU II

Le pourcentage d'animaux tués par la mouche de Goloubatz dans quelques départements de la Roumanie

	Départements	Chevaux Pertes %	Bovins Pertes %	Porcs Pertes %	Moutons Pertes %
1	Arges	1.90	3.22	1.65	0.17
2	Vâlcea	4.63	1.86	2.19	0.07
3	Muscel	2.10	1.62	0.45	0.02
4	Mehedinti	1.13	0.82	0.14	0.04
5	Dolj	0.79	0.80	0.15	0.02
6	Gorj	1.75	0.49	1.01	0,18
7	Timis-Torontal	0.21	0.95	—	—

Ainsi on voit par exemple dans ce tableau que le département d'Arges a perdu 3·22% de ses bovins et le département de Vâlcea a perdu 4·62% de ses chevaux et 2·49% de ses porcs.

Enfin dans le Tableau III ci-annexé nous avons donné d'après les évaluations officielles la valeur en lei des animaux tués.

Nous voyons de ce tableau que la valeur des animaux tués par la mouche de Goloubatz représente un capital d'environ 63,059,000 lei,* auquel il faut encore ajouter les dommages causés par la cessation du travail agricole, qui en est résultée.

* 800 lei (c.) = £1 0 0.

Le gouvernement roumain, désirant venir en aide aux fermiers les plus eprouvés, a mis à leur disposition une somme de 15,000,000 lei pour refaire leur bétail.

La manière dont la mouche de Goloubatz attaque les animaux et le moment de la journée où elle est agressive. A leur arrivée les Simulies se posent sur le corps des animaux, spécialement sur les muqueuses des orifices naturels et sur les parties les plus fines de la peau : lèvres, narines, yeux, oreilles, fanon, poitrine, face ventrale de l'abdomen, mamelles, organes génitaux, anus et la face interne des

Tableau III

La valeur en lei des animaux tués

Espèce animale	No. d'animaux tués	Valeur en lei
Chevaux	1.585	6.340.000
Ânes...	60	240.000
Bovins	10.592	52.960.000
Buffles	28	135.000
Moutons	915	366.000
Chèvres	460	184.000
Porcs	2.834	2.834.000
Total	16.474	63.059.000

membres. Les mouches de Goloubatz enfoncent assez profondément leur trompe dans la peau et y restent bien fixées jusqu'à ce qu'elles soient bien gorgées de sang, environ cinq minutes. Quelquefois elles deviennent tellement grosses qu'elles ne peuvent plus s'envoler.

Le plus souvent les bestiaux attaqués au pâturage s'agitent et s'enfuient à des dizaines de kilomètres. Quelquefois ils entrent dans l'eau pour se débarrasser des mouches. Les chevaux et les porcs rentrent dans leurs écuries. Les moutons et les chèvres ont moins à souffrir s'ils ne sont pas tondus. Nous avons observé aussi que les animaux à robe noire sont plus attaqués que les autres. Ainsi s'explique, je crois, l'affirmation de Tömösváry, que parmi nos animaux domestiques ce sont les buffles qui ont le plus à souffrir

pendant l'invasion de la mouche. Cet auteur a trouvé l'explication de ce fait en ce que les buffles étant des animaux indolents ne peuvent pas se défendre contre les mouches.

Presque dans tous les départements les mouches sont restées peu de temps dans la plaine, et se sont refugiées bientôt dans les forêts. Là, à l'abri des intempéries, elles ont pu vivre longtemps en faisant un grand nombre de victimes parmi les animaux.

En ce qui concerne le moment où les mouches sont agressives, nous avons observé qu'elles attaquent seulement pendant la journée, depuis le lever du soleil jusqu'à 10 heures du matin et dans l'après midi environ de 4 heures jusqu'au soir.

Pendant les heures les plus chaudes de la journée et pendant la nuit, les Simulies restent cachées dans des cavernes ou sous les feuilles des plantes.

Symptômes locaux. La piqûre des Simulies ressemble à celle de la puce. Le sang en suinte et quand plusieurs piqûres se trouvent dans la même région, celle-ci ressemble à une vaste plaie saignante. La peau d'un animal attaqué ainsi prend un aspect tacheté par les nombreuses piqûres, dont la plus grande partie confluent, formant des taches hémorragiques plus ou moins grandes. A l'endroit piqué se produit une induration sous la forme d'un petit bouton. Dans les cas des morsures multiples, les boutons confluent dans un grand oedème inflammatoire, dur et très douloureux, ce qui fait que les animaux se soustraient à la palpation. L'oedème de la tête chez le cheval donne l'impression d'un animal atteint de la Fièvre pétéchiale. L'oedème de l'abdomen, du fourreau et du pénis s'observe fréquemment chez le cheval, tandis que celui du fanon est commun chez le boeuf. L'oedème abdominal est quelquefois si grand qu'il donne l'impression d'un animal avec une hernie ventrale. Celui du pénis empêche la miction et cause la mort par l'urémie. Les animaux avec des oedèmes aux membres ont la marche difficile comme ceux atteints d'un rhumatisme articulaire.

Symptômes généraux. Les animaux gravement piqués manifestent les symptômes d'une asphyxie imminente : ils se meuvent très difficilement, ont la bouche entrouverte, la langue pendante, les yeux anxieux et la respiration suffocante ; enfin ils tombent et meurent en quelques heures (Triminoiu). Cela s'observe le plus souvent chez les bêtes surprises au travail par la mouche et qui

n'ont pas la possibilité de se défendre. Les animaux ayant souffert à cause des piqûres de la mouche, mais qui ne succombent pas dans les premières heures après l'attaque deviennent très irritables, ne mangent plus, et ont des frissons. La température est normale ou très peu élevée, la respiration accélérée et dispnéique, le pouls accéléré; on observe assez fréquemment un pouls veineux très manifeste. Les animaux deviennent apatiques, la respiration et le pouls sont presque imperceptible. La température descend sous la normale. Les victimes restent à peu près tout le temps couchés en découbitus sterno-abdominal (la tête tournée vers la queue comme les vaches atteintes de la Fièvre vitulaire (Velcov)). Ils conservent tranquillement cette attitude jusqu'à la mort qui survient après 6 ou 7 jours.

L'état des animaux chez lesquels la maladie évolue vers la guérison s'amende graduellement et revient à la normale après 7 ou 8 jours.

Lésions. A l'autopsie des animaux morts à la suite des piqûres de cette Simulie on a constaté des congestions et des dégénérescences des principaux viscères, spécialement du coeur, du foie et des reins.

Moyens pratiques pour prévenir à l'attaque des animaux. Dans les départements les plus souvent invadés, les moyens employés par la population rurale contre l'attaque de cet insecte sont les suivants :—

À l'arrivée de la mouche tous les animaux sont retenus pendant la journée dans des écuries sombres et on les conduit au pâturage et au travail seulement pendant la nuit. Pour empêcher la mouche d'entrer dans les écuries, on produit de la fumée en brulant du fumier dans le voisinage de la porte.

Si l'on est obligé de conduire les animaux au pâturage pendant la journée on produit autour d'eux de la fumée. De même on protège les animaux qui travaillent, en brûlant de la paille et du fumier dans un seau que l'on suspend à l'extrémité antérieur du timon.

Les fermiers préviennent encore à l'attaque de la mouche par des onctions faites sur les parties les plus vulnérables du corps de différentes décoctions et mixtures. Parmi les décoctions on emploie celles des feuilles d'absinthe, de noyer, de noisetier et même de tabac dans de l'eau ou du vinaigre (en proportion moyenne 1/10). On emploie aussi des mixtures de goudron minéral ou végétal avec

de l'axonge rance dans des proportions égales, ou même de l'axonge seule.

Il est intéressant de savoir comment les paysans de nos montagnes, spécialement ceux du département de Mehedinti, fabriquent le goudron végétal en utilisant le bois de *Pinus silvestris*. Dans ce but on fait dans la terre une fosse conique d'une profondeur d'un mètre, avec la base en haut, dont le fond communique avec l'extérieur par l'intermédiaire d'un petit canal (voir la figure du texte No. 1). Dans cette fosse on met des morceaux de bois de pin coupés en longueur, longs de 1 m. et larges de 4-5 cm. On range le bois dans la fosse obliquement, de sorte qu'il forme un cône qui dépasse la fosse d'un demi mètre. On couvre le bois qui reste en dehors avec de la terre en y laissant une petite ouverture à la partie supérieure par où on allume le bois. La combustion se fait ainsi de haut en bas.

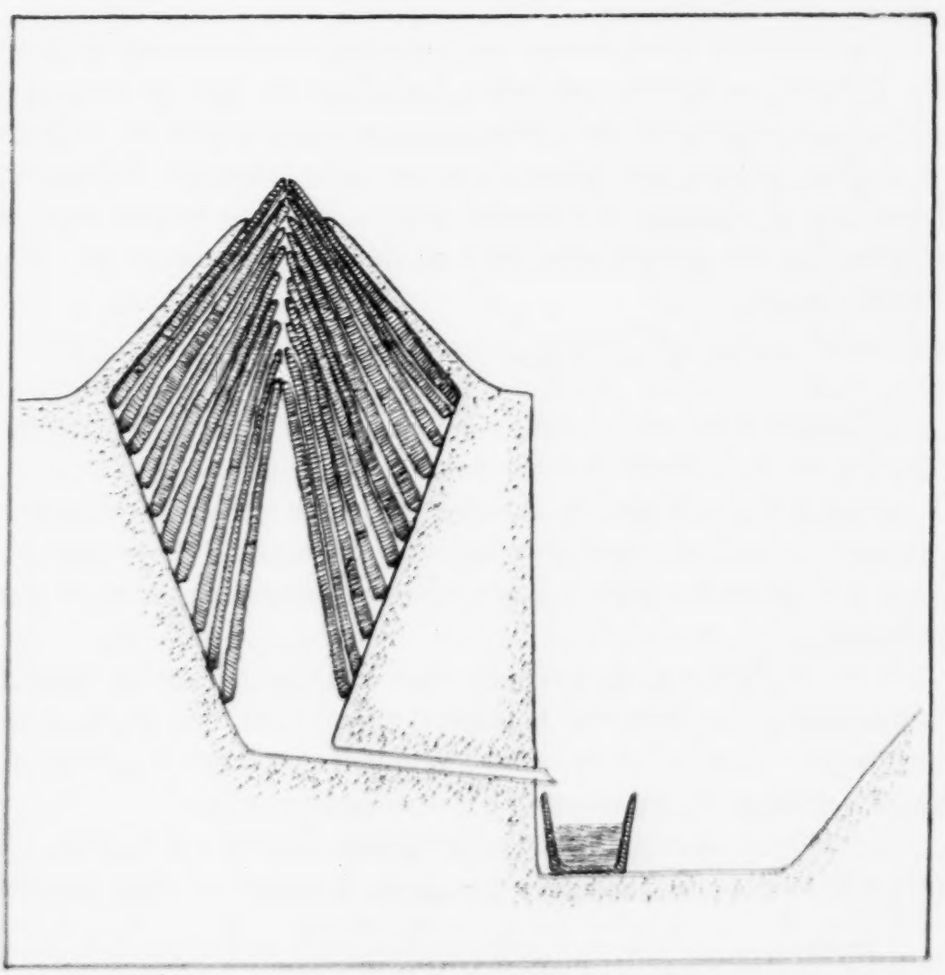


Fig. 1

La résine qui en distille, s'écoule par le canal inférieur de la fosse dans un vase. On mélange cette résine avant de l'employer, avec du lait et de la farine, en formant une pâte bien adhérente aux poils. Avec cette pâte on fait d'abord des onctions générales et on les répète de trois jours en trois jours sur les parties les plus fines et les plus vulnérables du corps.

Moyens curatifs. En ce qui concerne les moyens employés par nos fermiers pour guérir les animaux piqués par la mouche de Goloubatz il faut citer : Les frictions ou les compresses faites sur les régions malades avec de la saumure ordinaire ou de la saumure préparée avec une solution de vinaigre.

Les Médecins Vétérinaires ont employé des lotions ammoniacales pour neutraliser l'action du venin inoculé dans les plaies. De même on a recommandé des bains froids ou l'application de la glace pour combattre l'inflammation locale.

Comme médication interne on a employé avec beaucoup de succès les toniques cardiaques suivants : Infusions de café, de feuilles de digitales et injections de caféine. On pratique aussi des saignées.

Enfin, d'après les affirmations de nos Médecins Vétérinaires beaucoup d'animaux gravement piqués par cet insecte ont été abattus par les propriétaires et leur viande a été consommée sans aucun danger.

II. L'ATTAQUE CONTRE L'HOMME

L'homme aussi eut à souffrir cette année, à la suite de la grande invasion de la mouche de Goloubatz en Roumanie.

D'après les informations données par les Médicins les piqûres de ces insectes ont provoqué chez l'homme des manifestations morbides locales et générales, mais non des accidents mortels comme chez les animaux.

C'est à Monsieur le Docteur Cădere (Médecin de la ville de Câmpulung, département de Muscel) que nous devons une très intéressante étude clinique sur les accidents produits chez l'homme par les piqûres de cet insecte.* En voici un résume :

Symptômes locaux. La mouche pique l'homme à la tête, aux mains et aux pieds. Peu de temps après la piqûre, on a la sensation

* Des observations semblables ont été faites par nous cette année chez les paysans du département de Gorj, piqués par la mouche de Goloubatz (Photographies 6 et 7).

de cuisson et un peu plus tard la sensation d'une brûlure intense. De la plaie, qui est très fine s'écoule un véritable flot de sang, qui est en disproportion avec sa petitesse. Ce sang a perdu ses caractères normaux ; il est plus fluide et se coagule avec beaucoup de difficulté.

Quelques heures plus tard apparait autour de la piqûre une tache rougeâtre presque circulaire semblable à celle produite par la puce ou la punaise.

Après 12-24 heures ces taches s'aggrandissent et lorsqu'elles sont nombreuses s'unissent et forment des plaques d'un aspect erysipélateux. Ces plaques aux bords un peu élevés, d'une couleur de framboise, peuvent occuper des régions entières : face, bras, jambes. Celles-ci semblent alors atteintes d'une véritable lymphangite réticulaire. La région malade est plus sensible et plus chaude (37°5-37°8).

Aux endroits piqués il se forme de petits boutons, du sommet desquels s'écoule une sérosité trouble.

Dans une phase plus avancée et dans les cas plus graves, les lésions évoluent vers une infiltration oedémateuse de la peau, qui devient pâle jusqu'au blanc vitreux. En palpant la région malade on provoque des douleurs très vives et on constate que l'oedème est ferme et qu'il ne garde pas les emprintes des doigts, comme font les oedèmes ordinaires.

Autour des piqûres apparaissent ensuite de petites vésicules contenant une sérosité claire, disposées en demicercle ou cercle complet, semblables à celles qui entourent le centre d'une pustule maligne.*

Dans les cas légers, les vésicules se désèchent et disparaissent en 48-60 heures. Dans ceux plus graves, les vésicules se transforment en pustules, qui en 12-24 heures éclatent et donnent naissance à des ulcérations d'un aspect sale. La plaque centrale se mortifie et s'élimine sous la forme d'une escarre mesurant 2-6 cm. La régénération des tissus se fait lentement (10-15 jours) en produisant une cicatrice blanchâtre.

Les ganglions lymphatiques prennent part au procès inflammatoire dans la mesure des altérations locales. Ainsi dans les cas légers ils sont seulement sensibles, tandisque dans les cas graves ils sont hypertrophiés et très douloureux.

* Des cas semblables ont été observés par Mr. le Dr. Drăghicescu, Médecin de la ville de Târgul Jiu.

Symptômes généraux. Ceux-ci varient d'après la gravité des lésions locales. Quand ces lésions sont peu importantes, on observe seulement un état d'indisposition, d'agitation et un fourmillement dans tout le corps ; quand elles sont plus graves la température baisse (36°), on constate un état d'algidité avec refroidissement des extrémités, un pouls mou, de la diarrhée et une oligurie passagère.* Au fur et à mesure que les altérations locales cèdent, les symptômes généraux disparaissent et les malades guérissent.

Traitement. Localement on a appliqué des onctions avec liniment ammoniacal.† Comme médication interne on a administré des diurétiques, des toniques cardiaques et du chlorure de calcium dans les cas accompagnés d'hémorragies plus abondantes.

Prophylaxie : Celle-ci consiste principalement dans la protection contre les piqûres de la mouche, des régions attaquables, en utilisant de différents moyens comme un masque ou un linge quelconque pour la figure, des gants et des chaussures. De même on peut prévenir à l'attaque par l'onction des régions énumérées avec des mélanges de substances grasses ou huilleuses, avec de l'ammoniaque ou des essences volatiles.

En terminant cette note nous voulons mentionner que la question de la mouche de Goloubatz a été étudiée jusqu'à présent spécialement par Schönbauer, Tömösváry et Aigner-Abafi en Hongrie ; par Moga et par Leon en Roumanie ; par Medovici et par Georgévitch en Jugoslavie ; par Dobrev et par Gheorghieff en Bulgarie.

* D'après une communication, qui nous a été faite par Mr. le Dr. Draghiescu, les malades dans les cas plus graves sont obligés d'interrompre leur occupation pendant 8-12 jours.

† Le Dr. Draghiescu nous a informé qu'il a employé avec de bons résultats des compresses ou des lotions avec du vinaigre sur les régions malades.

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EXPLANATION OF PLATE XVIII

FIG. 1. Les ruines du château de Goloubatz dans la Jugoslavie ; cliché pris sur la rive roumaine du Danube, à Moldova Veche, village situé vis-à-vis de ce château dans le département de Caras-Severin du Banat.

FIG. 2. La grotte " Gaura cu musca " (la grotte à la mouche) dans les montagnes du Banat, près du village de Coronini, sur la rive gauche du Danube.

PLATE XVIII

FIG. 1

FIG. 2

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EXPLANATION OF PLATE XIX

FIG. 1. Paysans roumains avec la charrue au travail du champ pendant l'invasion de la mouche de Goloubatz. On voit deux seaux attachés au timon, produisant de la fumée par la combustion du fumier.

FIG. 2. Boeuf avec l'oedème du fanon, produit par des piqûres de la mouche de Goloubatz.

FIG. 1

FIG. 2

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EXPLANATION OF PLATE XX

FIGS. 1 and 2. Paysannes du département de Gorj, piquées par dès Simulies. On voit des ecchymoses, des boutons et des oedèmes de la jambe.

FIG. 3. Peau de boeuf avec des piqûres de la mouche de Goloubatz.

PLATE XX

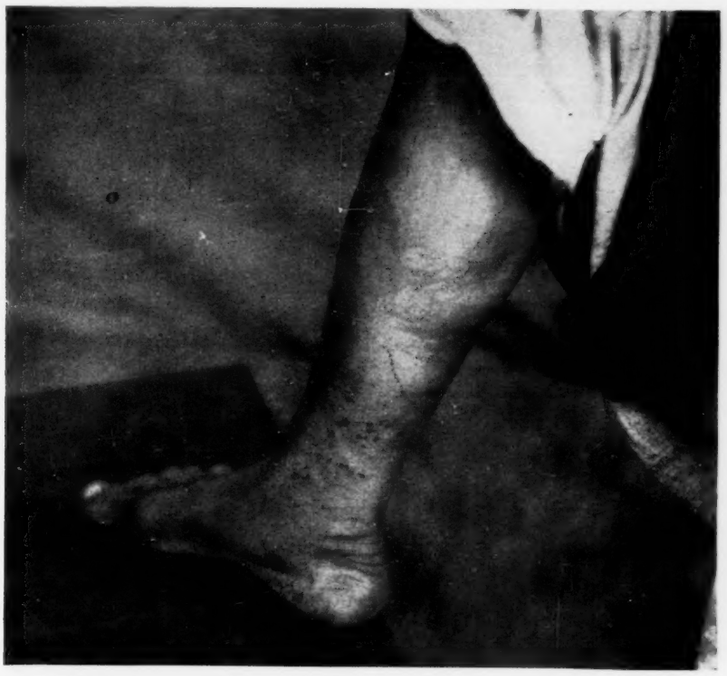

FIG. 1

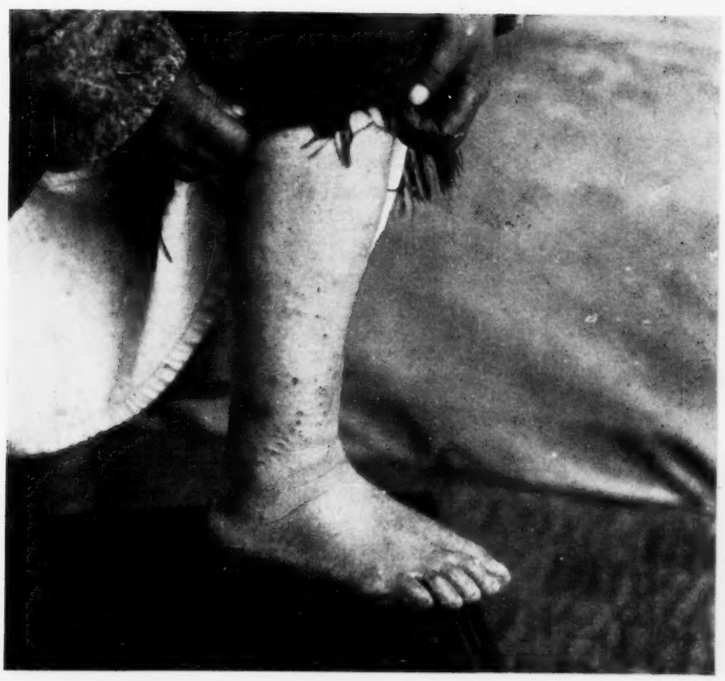

FIG. 2

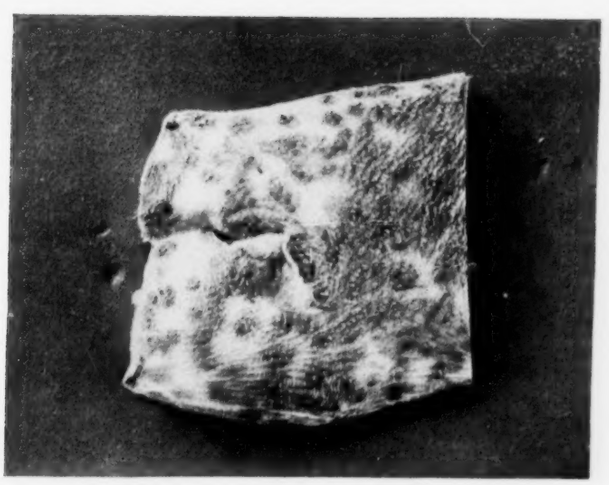

FIG. 3

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THE SOURCE OF SOUTH AFRICAN TREMATODES

BY

F. G. CAWSTON, M.D. (Cantab.).

(*Received for publication* 4 *July*, 1924)

According to the literature that was available up till 1915, the only fresh-water snail that was held to be responsible for the development of trematode parasites of man and beast in South Africa was a minute species which had been shown to be responsible for Fasciola disease in other lands, namely *Limnaea truncatula*. I have examined some of the very few examples of this rare snail from the Transvaal ; but, as it had not been found infested with cercariae in South Africa, its existence may be regarded as of very little practical importance fron a disease-prevention point of view. The shell of *L. truncatula* is about 5 mm. in length. I have repeatedly shown that *Limnaea natalensis* is the usual carrier of the Fasciola parasites and its shell is probably the largest of its kind in the Union. I have collected an example from the Umhlangana that was fifteen-sixteenths of an inch in length. An occasional carrier of fasciolae and the usual carrier of the various schistosomes in South Africa, *Physopsis africana*, is about the same size. I found an example in the Durban suburbs that was 20 mm. in length. The shell closely resembles that of *Isidora globosa*, Morelet, of which I have obtained examples up to 19 mm. in length from Lourenço Marques. *Isidora tropica*, a common carrier of amphistomes and an occasional carrier of schistosomes, is common all over the Union. I have collected examples measuring 16 mm. in length, both from Schuttes' Draai, O.F.S., and from Durban. Another common carrier of trematodes is *Planorbis pfeifferi* and I have an example from the Umbilo 15 mm. in diameter.

We may say that the commoner fresh-water snails of South Africa that are responsible for trematode parasitic diseases, possess shells which measure from 15 to 20 mm. in length. However, *Melanoides tuberculata*, which is heavily infested at the Natal Coast, has an operculated shell which at Durban measures 31 mm. in length.

There are, however, a large number of smaller South African shells which may be overlooked in examining collections of semi-stagnant water for the possible presence of intermediary hosts. First in importance is *Isidora forskali* whose shell is about the size of *Limnaea truncatula* but dextral. In the smaller spruits all over the Union this common fresh-water snail is infested with amphistomes. These common parasites of cattle and sheep cause so few symptoms that little attention has been paid to their life-history. There is an incredible number of very minute shells to be found attached to reeds and floating wood which have escaped the attention they deserve as carriers of disease, probably because they usually reach a diameter of not more than 5 mm. and because their shells are so fragile. The largest genus of the *Ancylidae* is *Burnupia* and the shells of the various species measure from 3 to 9 mm. in length, whilst the *Ferrissia* and *Gundlachia* are seldom more than 4 mm. in length. I have repeatedly examined these snails for parasites, but could find no trace of infection, until in October, 1923, I found cercariae with divided tails in *Burnupia trapezoidea* (Bttg.) at Schuttes' Draai, O.F.S. Dr. E. C. Faust is describing these monostomes for me; they are about the same size as *Schistosoma*. Similar monostomes were present in *Burnupia capensis natalensis* from a small spruit at Escombe, Natal, in June, 1924. The head was 0·13 mm. in length and both tail and prongs 0·175 mm. in length, making a total length of this narrow cercaria of 0·48 mm. The shell from which these cercariae escaped was only 4 mm. in length. They were sporocyst-produced. In May, 1924, I found redia-produced distomes in two distinct species of *Burnupia* at Avoca, Natal. *Burnupia stenochorias* M. and P., *B. capensis natalensis*, Walker, and *B. caffra*, Krauss, are all present in this locality. The shell of one infested *Burnupia* was only 4 mm. in length and none were more than 6 mm. in length. The cercaria possessed a chain of cystogenous particles on each side of the body and measured 0·6 mm. in total length.

It is very difficult to extract the animal from these smaller shells for the examination of cercariae and, having convinced oneself of the presence of cercariae, to keep the entire shell for identification. However, I obtained numerous *Ferrissia* (?) *connollyi* from a small pool at Malvern, Natal, said to contain tortoises, and, having

extracted the animal from a shell only 3 mm. long by means of a dental needle, found it infested with *Megalodiscus sp.* (?) measuring 0·525 mm. in length and about 0·4 mm. in breadth. This pool also contained an interesting *Gundlachia* besides *Isidora forskali.*

There is a small water-lily pool in the Botanic Gardens at Durban in which are kept gold-fish and ' millions.' Attached to the leaves of the water-lilies are a few examples of *Limnaea natalensis,* the common intermediary host in this country for *Fasciola gigantica,* as well as examples of *Ferrissia natalensis* and *F. burnupi.* Microscopic examination of these *Ferrissiae* revealed, in a large proportion of snails dissected, a styletted cercaria about 0·26 mm. in total length, the undivided tail being half the length of the body. One infected example measured only 2 millimetres in length. The identity of this distome is being investigated ; but its presence in so minute a shell emphasises the importance of examining shells so minute as those of *Ferrissia* and *Gundlachia* for the possible presence of trematode parasites. The *Limnaeae* were free from infestation.

Various cercariae have been obtained from *Planorbinae* no more than 4 mm. in diameter and *Segmentina planodiscus,* whose shell is only slightly larger, harbours a rather large eye-spotted schistosome at the Durban Country Club.

South African trematodes develop more frequently in fresh-water snails whose shells are from 15 to 20 mm. in length ; but those species whose shells are no more than 4 to 6 mm. in diameter are occasional carriers of these parasites and prophylactic measures must be directed towards the possible eradication of all varieties, now that, for the first time, trematodes have been isolated from *Ferrissia* and from more than one species of *Burnupia.* In a young country there is a tendency to overlook the large amount of apparently unnecessary and arduous work that must be carried out before one can speak with confidence in regard to those measures which farmers must adopt, if they are to keep their farms free from parasitic diseases. Some years ago I urged the Union Government to introduce domesticated duck in order to keep down the number of Bilharzia-producing snails along the course of the Umbilo river at Pinetown and Sarnia. Instead of carrying out this suggestion, the Government investigated the desirability of introducing otters

into the river. As a matter of fact otters feed on crabs and, as crabs feed readily on snails, otters would indirectly have helped to increase the number of fresh-water snails in this badly-infested river.

Careful study of Bilharzia infection along natural history lines in Natal, over a period of several years, confirms the opinion of other workers and convinces me that our efforts must be directed towards :—

(1) The introduction of domesticated duck wherever possible.

(2) The use of lime or of Ross's larvicide in small collections of water.

(3) Drying up collections of water, at any rate for a week at a time, and this would appear to be the best method of dealing with the minuter fresh-water snails.

(4) Though it has only a limited application, at seaside resorts, in Natal we might follow nature's example where the inflow of sea-water into the lagoons destroys those fresh-water snails that are continually being carried down to the lagoons.

(5) The collection and destruction of such species as *Melanoides tuberculata*, whose operculated shell is to be found in incredible numbers in the Durban water-cress beds, and has been shown to be too stout for ducks to eat.

LOCOMOTOR ATAXIA WITH CHARCOT'S JOINT DISEASE IN A NEGRO

BY

J. L. PAWAN, M.B., Ch.B. (Edin.), D.P.H. (Lon.),

GOVERNMENT BACTERIOLOGIST, TRINIDAD, B.W.I.

(*Received for publication* 31 *July*, 1924)

PLATE XXI

History. Patient a negro, 58 years of age, was born in Trinidad, and has lived there all his life. When 19 years old he developed a chancre on his penis which healed readily with local application. Seven years later he married ; a year after marriage his first child was born. His wife gave birth to eleven children all of whom died either a few days or a few months after birth except the fifth and seventh who are alive and well.

He was apparently in good health up to twelve years ago, *i.e.*, twenty-seven years after the appearance of the chancre, when he began to suffer from (1) periodic attacks of sharp shooting pains about his knees and legs with a tendency to fall when walking ; (2) hot burning sensations about his body ; (3) difficulty of micturition ; (4) tenderness and pains about his knee-joints which gradually increased in size. The pains about his knees persisted for two years and the difficulty with micturition for six years. He has been impotent for the last eight years.

Clinical Signs. A fairly well-nourished man with no evidence of mental impairment, but with characteristic knee lesions. There is relaxation of the ligaments and other soft parts of the knee-joints with enlargements of the lower end of the femur and the upper end of the tibia and loss of apposition of the contiguous surfaces of the bones, so that the upper ends of the tibiae are thrown backwards and outwards. There is some atrophy of the muscles of the legs and thighs. There is palpable thickening of the synovial membranes, but no increase in the fluid. There is an abundance of rotatory movement at the knees with flail-like movements of the

legs and a remarkable absence of pain and resistance with these movements. Flexion at the knee-joint is complete, but extension is reduced by about one quarter.

These changes are more marked on the left than on the right side.

Sensation of heat is normal, that of cold is impaired.

The knee jerk and ankle jerk are absent. There is no ankle clonus.

The Babinski is doubtful. There is incoordination of the lower extremities, but not of the upper. The hand grip is firm.

Argyll-Robertson pupil is present. Rombergism is present and well-marked. The patient's walk, though not typically ataxic, is characteristic. He rests on his stick, keeping his eyes centred on the ground and his body thrown forward, with his legs held slightly apart. The feet are brought down with a stamp, the heels touching the ground first, or the whole foot comes in contact with the ground.

The blood Wassermann by Harrison's method gave a positive reaction (++++). There is no gastric, rectal or other crisis and his only complaint is in connection with the discomfort and inconvenience due to the disorganisation of his knee-joints.

EXPLANATION OF PLATE XXI

Locomotor Ataxia with Charcot's Joint Disease in a Negro.

Fig. 1. Anterior view.

Fig. 2. Posterior view.

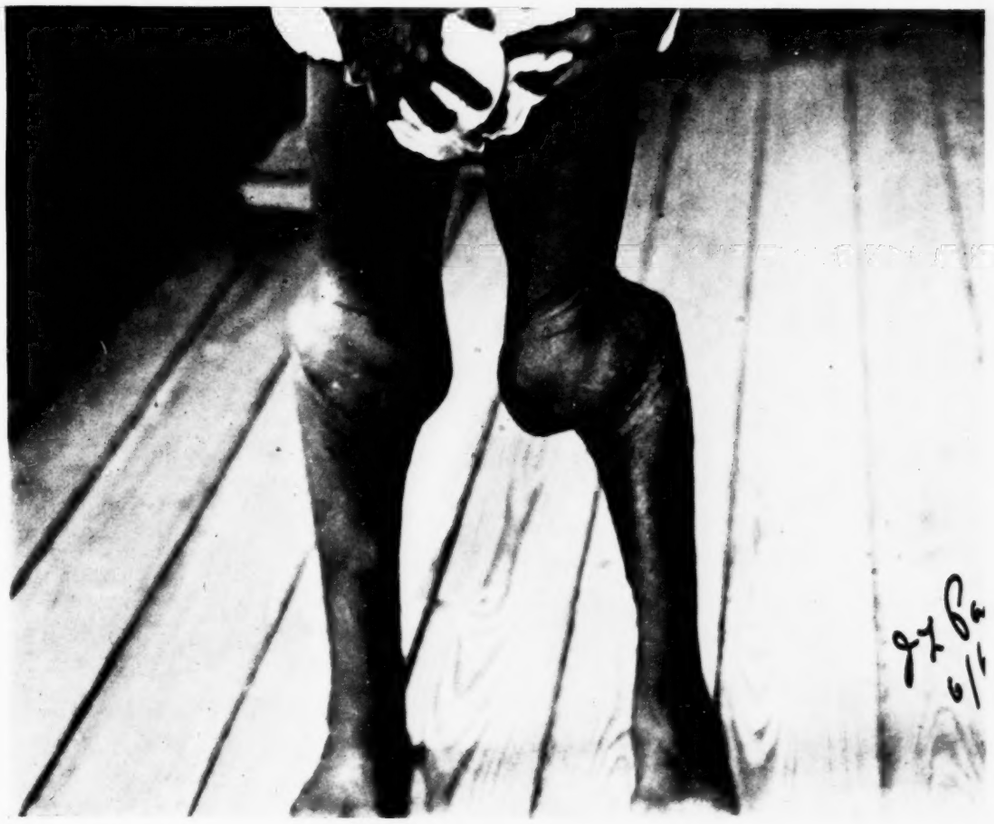

FIG. 1

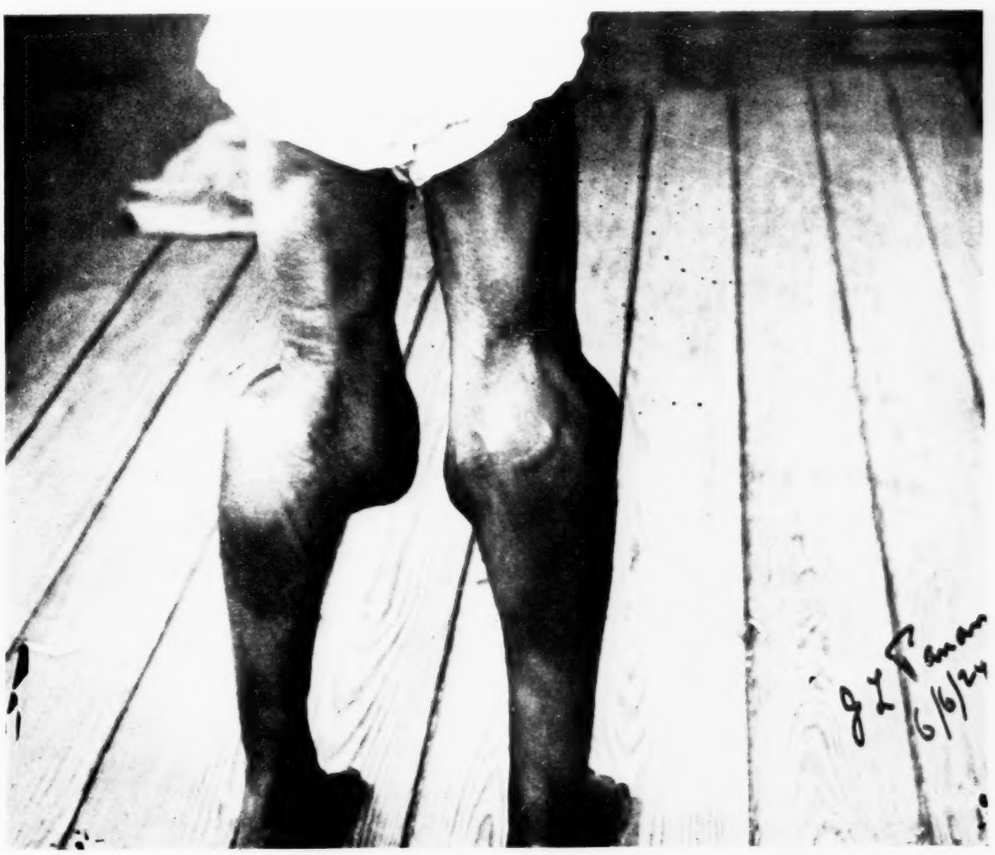

FIG. 2

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THE VALUE OF URINARY EXAMINATIONS IN THE DIAGNOSIS OF MALARIA

BY

R. M. GORDON

From The Sir Alfred Lewis Jones Research Laboratory, Freetown

(*Received for publication* 5 *August*, 1924)

INTRODUCTION

The work which follows was commenced in England and continued in West Africa. It was undertaken with the object of ascertaining whether the more commonly employed urinary tests and examinations have any value as aids in the diagnosis of malaria ; if they have then their importance is obvious, more especially in those only too frequent cases in which the blood is negative and the patient gives a history of having had ' fever ' a few days previously, for which he took quinine. The urines were examined for urobilinogen, urobilin, albumin, casts, indican, bile pigments, and urea percentage.

Urobilinogen and Urobilin. Considerable difference of opinion exists regarding the importance attachable to the presence of these pigments in the urine. Plehn (1908) believes urobilinuria to be a point of great diagnostic value, but Surveyor (1910), while working in India, was unable to confirm Plehn's observations. Acton and Knowles (1913) state

' Urobilin is not found in the urine of healthy persons, but is found in various fevers, *e.g.*, pneumonia, and the late stages of typhoid and Malta fevers, etc.'

In their summary they state

' The presence of urobilin in the urine in large quantities indicates that haemoglobin is being destroyed. The blood destruction, except in certain well-defined diseases, is due to the destruction of erythrocytes by malarial parasites. If there is no fever present the patient is in the later stages of the disease.'

Acton and Knowles made no quantitative tests and, judging by the context, the expression ' large quantities ' simply means a well-marked fluorescence with Schlesinger's solution. Simpson (1910) examined twenty-two cases of malaria and estimated the urinary urobilin quantitatively on several occasions in each individual ; he found that in simple tertian malaria the output of

urobilin is never greater than in other diseases such as pneumonia. He proceeds :

> ' In malignant tertian malaria (*P. falciparum*) a different picture is obtained ; definite urobilinuria sets in very shortly after the onset of the pyrexia, reaches its height on the second or third day and then slowly diminishes ; the normal level of excretion is reached in ten days unless a fresh paroxysm intervenes. Sometimes the increased output continues for a longer period (*e.g.*, Case 20) and in old standing cases with chronic malarial anaemia there may be a continuous raised urobilin output even in the absence of pyrexia (Case 19).'

On looking up Case 20, we find that on seven out of twenty-eight examinations the urobilin output is marked as ' 0,' and in Case 19, referred to in the text as ' Continuous raised urobilin output,' three out of eighteen examinations are marked ' 0.' Ballerstedt (1924) states in his summary that urobilin persists in the urine of convalescent malarias and for a long time after the cessation of all attacks, but is careful to point out that slight urobilinuria may be found in healthy people ; he notes that when the patient's temperature has been normal for some time the urobilinogen test is usually negative while the urobilin remains positive. Sorensen (1914) does not believe in the persistence of urobilin except for two to four days after the subsidence of fever, ' though its reappearance invariably presages a relapse.' Di Pace (1923) after the examination of some one hundred and eighty malarial urines reaches the following conclusions regarding urobilinuria : (1) It is present in all acute malarias with parasites and symptoms still present. (2) Frequent in patients who had an attack of malaria two to three months previously and in whom parasites are still present. (3) Rare in cases with a history of malaria more than three months previously clinically cured, but who show parasites after the administration of strychnine. (4) Extremely rare in latent malarias that are absolutely without symptoms. Di Pace unfortunately appears to have used no controls.

Albumin and casts. Nephritis as a concomitant or complication of malaria is referred to by almost all writers on malaria. Levy Moise (1923) in a paper reviewing previous workers' figures, points out its great frequency. Moise examined seventy-six cases of malaria and found albumin in 27.6 per cent. and casts in 25 per cent. Gordon (1923a) found nephritis in ten out of sixteen cases of malaria examined in England.

Indican, bile pigments, and percentage of urea. These will be dealt with later.

TESTS EMPLOYED IN THE URINARY EXAMINATIONS

All urines were examined as soon after passing as possible and in hospital cases the first urine passed in the morning was always tested.

Urobilinogen. Two drops of Ehrlich's aldehyde reagent, which consists of a 3 per cent. solution of paradimethylaminoazobenzaldehyde in 50 per cent. hydrochloric acid, were added to five c.cs. of urine and the tube gently warmed to blood heat ; the appearance of a rose pink to scarlet colour was regarded as positive. MacCormac and Dodds (1923) when describing this reaction state ' This test when positive demonstrates the presence of a pathological amount of urobilinogen.'

In order to prevent the conversion of urobilinogen to urobilin the urine to be tested was exposed to light as little as possible ; Graham (1911) in a paper on the effects of quinine on the excretion of urinary pigments, states that he placed ten hour samples of urine in a well-lighted window for two hours in order to ' Ensure the complete conversion of urobilinogen to urobilin.' In connection with this it may be remarked that the present writer frequently noted that a urine giving a positive urobilinogen reaction was still positive after eight hours exposure to full daylight.

Urobilin. Schlesinger's test was used, twenty c.cs. of urine were acidulated with acetic acid and the urobilin extracted by gently inverting with five c.cs. of amyl alcohol. (The mixture must be made gently or an emulsion will result.) The amyl alcohol was then pipetted off and half a c.c. of a 10 per cent. solution of zinc chloride in absolute alcohol added to it ; the appearance of distinct fluorescence and a zinc-urobilin band in the spectrum were taken as positive ; the spectroscopic result is important, as Ballerstedt (1924) has pointed out that the blue fluorescence of quinine in the urine may influence the typical green fluorescence of urobilin.

Albumin. Sulphosalicylic acid was prepared by dissolving thirteen grammes of salicylic acid in twenty grammes of sulphuric acid, by warming, and after cooling, adding sixty-seven c.cs. of water (Cole (1920)). If no ring resulted on the addition of urine, albumin was regarded as absent ; it was found, especially amongst West African natives, that mucin was frequently present and gave a ring indistinguishable from albumin and it was found necessary to

exclude this by testing for increased opacity on the addition of dilute acetic acid.

Indican. To five c.cs. of urine in a test-tube were added one large drop of 5 per cent. potassium chlorate, then five c.cs. of strong hydrochloric acid, followed by five c.cs. of chloroform, the contents being mixed by inverting the closed test-tube a couple of times. Definite blue colouration in the separated chloroform was regarded as positive.

Bile pigments. Various reagents were tried and Fouchet's test, as described by MacCormac and Dodd (1923) for testing serum, was found the most satisfactory ; to a small quantity of urine was added an equal quantity of the following reagent, trichloracetic acid five grammes, water twenty c.cs. and 10 per cent. ferric chloride solution two c.cs. ; bile pigments were indicated by the appearance of a bright green precipitate.

Urea percentage. This was roughly estimated in the usual way with a ' Southall ureometer.'

RESULTS

The following two points must be taken into consideration when studying the figures that follow :

(1) Amongst the cases examined in England the heading ' Malaria. Parasites present,' means that parasites were actually present in the blood at the time that the urine was passed. In the cases examined in West Africa, as it was not always possible to examine the urine on the same day as the positive blood film was obtained, all that can be said with certainty is that parasites were present within a few days previous to the urinary examination.

(2) No distinction between the different species of malaria parasites is made in the tables ; actually when compiling the figures the different urinary findings accompanying malignant tertian, benign tertian, or quartan malaria were recorded, but as the differences found were of a trivial character no record of them has been retained in the paper.

TABLE I.

Showing the frequency of urobilinogen in the urine of 200 individuals in England and Sierra Leone.

	ENGLAND				FREETOWN, SIERRA LEONE							
	Europeans				English residents				Natives			
	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites present	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites present	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites present
Number of cases examined ...	18	30	4	19	18	10	...	28	33	24	8	8
Percentage positive ...	11	30	50	84	17	0	...	39	6	33	62	87

Remarks. In none of the cases recorded above was urobilinogen detected without urobilin also being found.

TABLE II.

Showing the frequency of urobilin in the urine of 200 individuals in England and Sierra Leone.

	ENGLAND				FREETOWN, SIERRA LEONE							
	Europeans				English residents				Natives			
	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites present	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites present	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites present
Number of cases examined ...	18	30	4	19	18	10	...	28	33	24	8	8
Percentage positive ...	11	53	75	95	39	10	...	89	12	50	87	87

Remarks. Under the heading 'Mixed hospital cases' there are included four cases of amoebic dysentery; one of these gave a positive urobilin reaction. A case which had been operated on for amoebic liver abscess twelve months previously and which still showed signs of hepatitis was also tested, but gave a negative reaction. In connection with this it may be noted that Grall, as quoted by the *Annals of the Clin. Laboratories* (1924), 'Considers that in a colonial subject abundant urobilinuria ought forthwith to make one suspicious of amoebic hepatitis.'

TABLE III.

Showing the frequency of albumin in the urines of 202 individuals in England and Sierra Leone.

	ENGLAND				FREETOWN, SIERRA LEONE							
	Europeans				English residents				Natives			
	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites present	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites present	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites present
Number of cases examined ...	18	30	4	21	18	10	...	28	33	24	8	8
Percentage positive ...	0	37	75	71	5	20	...	63	24	42	62	36

Remarks. The large number of albuminurias among normal natives in Sierra Leone is probably explained by the fact that the individuals examined were convicts, many of whom were known to be suffering from chronic gonorrhoea.

TABLE IV.

Showing the frequency of casts in the urine of 200 individuals in England and Sierra Leone.

	ENGLAND				FREETOWN, SIERRA LEONE							
	Europeans				English residents				Natives			
	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites present	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites present	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites present
Number of cases examined ...	18	30	4	19	18	10	...	28	33	24	8	8
Percentage positive ...	0	17	25	53	0	10	...	25	3	29	12	12

Remarks. The low percentage of malaria cases showing casts amongst the people examined in West Africa, as compared with those in England, may be due to their having received early and efficient quinine treatment, while the cases examined in England had suffered from a lack of thorough treatment during a long sea voyage.

TABLE V.

Showing the frequency of indican in the urine of 202 individuals in England and Sierra Leone.

	England				Freetown, Sierra Leone							
	Europeans				English residents				Natives			
	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites Present	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites Present	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites Present
Number of cases examined ...	19	29	4	21	18	10	...	28	33	24	8	8
Percentage positive ...	21	41	0	43	11	10	...	25	6	21	0	12

Remarks. Judged on these figures indicanuria would appear to be less frequent in the tropics than in England; for further European figures see Gordon (1923b).

TABLE VI.

Showing the frequency of bile pigments in the urine of 200 individuals in England and Sierra Leone.

	England				Freetown, Sierra Leone							
	Europeans				English residents				Natives			
	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites Present	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites Present	Normal	Mixed hospital cases	Clinical malaria	Malaria. Para ites Present
Number of cases examined ...	18	30	4	19	18	10	...	28	33	24	8	8
Percentage positive ...	0	7	0	0	0	0	...	0	0	0	0	0

Remarks. From this it will be seen that bile pigments were only detected in the urine of two out of 200 individuals examined; in both these cases the patient was suffering from well-marked obstructive jaundice.

TABLE VII.

Showing the frequency of urea above three per cent. in the urines of 57 individuals in England.

	England			
	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites present
Number of cases examined	15	22	4	16
Percentage positive	7	9	0	56

TABLE VIII.

Showing the results of urinary examinations in three cases of general paralysis of the insane, before, during, and after an attack of simple tertian malaria. (Note—'+' signifies presence of, 'o' absence of, and '—' no examination made.)

CASE 1. Anophelines infected with *P. vivax* fed on patient, 31.12.23.

	January																	February				
Date, 1924	8	9	10	11	12	13	14	16	18	19	21	23	25	27	28	29	31	2	4	6	8	1 and 1
First temperature ...	+	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Parasites	o	o	+	+	+	+	+	+	+	+	+	+	+	+	+	+	o 30=o	o	o	o	o	
Quinine Grains 30...	o	o	o	o	o	o	o	o	o	o	o	o	o	o	+	+	o	o	o	o	o	
Urobilinogen ...	o	o	+	+	+	+	o	o	+	+	+	+	+	+	—	+	o	+	o	o	o	
Urobilin	o	o	+	+	+	+	+	+	+	+	+	+	+	+	—	+	+	+	o	o	o	
Albumin	o	o	o	+	+	+	+	+	+	+	+	+	+	+	—	+	+	+	+	o	o	
Casts	o	o	o	o	+	+	+	+	+	+	+	+	+	+	—	+	+	+	+	+	o	
Indican	o	+	+	+	+	+	+	+	o	+	+	+	o	o	—	+	o	o	+	o	+	
Bile pigments ...	o	o	o	o	o	o	o	o	o	o	o	o	o	o	—	o	o	o	o	o	o	
Urea above 3% ...	—	—	o	+	+	+	+	+	+	+	+	o	o	o	—	o	o	o	o	o	o	

TABLE VIII (cont.).

CASE 2. Anophelines infected with *P. vivax* fed on patient, 21.2.24.

	FEBRUARY			MARCH																APRIL
Date, 1924	21	24	27	1	4	6	8	10	12	14	15	16	18	20	22	24	26	28	30	13
First temperature	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Parasites	o	o	o	o	o 5=o	+	+	+	+	+	+	+	o	o	o	o	o	o	o	o
Quinine grains 30	o	o	o	o	o	o	o	o	o	o	+	+	o	o	o	o	o	o	o	o
Urobilinogen	o	o	o	o	o	o	o	o	o	+	—	+	+	o	o	o	o	o	o	o
Urobilin	o	o	o	o	o	+	o	o	+	+	—	+	+	+	o	o	o	o	o	o
Albumin	o	o	o	o	o	o	o	+	+	+	—	+	+	+	+	o	o	o	o	o
Casts	o	o	o	o	o	o	+	+	+	+	—	+	+	+	+	o	o	o	o	o
Indican	o	o	o	o	o	o	o	o	o	o	—	o	o	o	o	o	o	o	o	o
Bile pigments	o	o	o	o	o	o	o	o	o	o	—	o	o	o	o	o	o	o	o	o
Urea above 3%	o	o	o	o	o	o	+	+	o	o	—	+	o	o	o	o	o	o	o	o

TABLE VIII (cont.).

CASE 3. Anophelines infected with *P. vivax* fed on patient, 22.2.24. Note—Slight nephritis was present before infection.

	FEBRUARY			MARCH																APRIL	
Date, 1924	22	25	28	2	5	7	8	9	10	12	14	16	18	20	22	24	26	28	30	10	18
First temperature	...	...	...	...	...	+	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Parasites	o	o	o	o	o	o	o	o	+	+	+	+	+	+	+	+	+	+	o	o	o
Quinine grains 10	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	+	+	+	o
Urobilinogen	o	o	o	o	o	—	+	—	+	+	o	o	+	+	+	+	+	+	+	o	o
Urobilin	o	o	o	o	o	—	+	—	+	+	+	+	+	+	+	+	+	+	+	o	o
Albumin	Tr.	Tr.	Tr.	Tr.	Tr.	—	+	—	+	+	+	+	+	+	+	Tr.	Tr.	Tr.	Tr.	o	o
Casts	+	o	o	+	o	—	+	—	+	+	o	+	+	o	o	o	o	o	o	o	o
Indican	+	o	o	+	o	—	+	—	o	o	o	o	o	o	o	o	o	o	o	o	o
Bile pigments	o	o	o	o	o	—	o	—	o	o	o	o	o	o	o	o	o	o	o	o	o
Urea above 3%	+	o	o	o	o	—	o	—	o	o	o	+	o	o	o	o	o	o	o	o	o

Remarks. In Cases 1 and 2 urobilinogen and urobilin first appeared either on the same day, or else later than, the parasites; in Case 3 both urobilinogen and urobilin preceded the parasites by two days, in all three cases these substances persisted for one to five days after the blood had become negative. Albumin and casts appeared in Cases 1 and 2—and were increased in Case 3—within three days of the first positive blood finding, and persisted in Cases 1 and 2 for about a week after the disappearance of parasites; in Case 3 it will be noted that casts ceased to appear in the urine for some days prior to the blood becoming negative.

The number of days during which urobilinogen, urobilin, albumin and casts persisted in the urine after parasites had disappeared may also be noted in the following additional cases. *Case A.* Urobilinogen less than four days, urobilin more than four days, albumin more than four days, casts less than four days. *Case B.* Urobilinogen less than four days, urobilin less than four days, albumin more than four days, casts less than four days. *Case C.* Urobilinogen, urobilin, and albumin less than ten days, casts more than ten days. *Case D.* Urobilinogen and urobilin less than seven days, albumin or casts did not appear. *Case E.* Urobilinogen and urobilin more than nine days, albumin and casts less than nine days.

TABLE IX.

Showing the results of urinary examinations during pyrexial and apyrexial periods in a case of lymphadenoma. Note—The patient had never been abroad.

	JANUARY			FEBRUARY															
Date, 1924	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	17	18
Temperature	98	100	101	102	102	103	98	98	98	98	98	98	98	98	98	101	1
Urobilinogen	—	o	—	+	—	—	o	—	o	—	+	—	+	—	o	—	+
Urobilin	—	+	—	+	—	—	o	—	o	—	+	—	+	—	o	—	+
Albumin	—	o	—	o	—	—	o	—	o	—	o	—	o	—	o	—	o
Casts	—	o	—	o	—	—	o	—	o	—	o	—	o	—	o	—	o
Indican	—	o	—	+	—	—	o	—	+	—	+	—	+	—	o	—	o
Bile pigments	—	o	—	o	—	—	o	—	o	—	o	—	o	—	o	—	o
Urea above 3%	—	o	—	o	—	—	o	—	o	—	o	—	o	—	o	—	o

Remarks. Note the irregularity of the appearance of both the urobilinogen and the urobilin and that they do not always correspond to the pyrexial periods.

TABLE X.

Showing the frequency of a combination of urobilin, and albumin (with or without urobilinogen, casts, indican, bile pigments, or raised urea percentage), in the urine of 200 individuals in England and Sierra Leone.

	ENGLAND				FREETOWN, SIERRA LEONE							
	Europeans				English residents				Natives			
	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites present	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites present	Normal	Mixed hospital cases	Clinical malaria	Malaria. Parasites present
Number of cases examined ...	18	30	4	19	18	10	...	28	33	24	8	8
Percentage positive ...	0	13	50	84	0	0	...	46	0	25	62	37

CONCLUSIONS

As regards positive findings, it can at once be stated that no individual test was found to have any real value as an aid in the diagnosis of malaria, one and all occurring with considerable frequency not only in diseases other than malaria but also in the normal individual, as is clearly shown in Tables I to VII ; so far, therefore, as any single test is concerned the results obtained are in accordance with the views expressed by Lane (1923) who remarks

'There emerges from all this the conclusion, old but needing as much emphasis as ever it did. In any particular case the finding of parasites is the only justification for a positive diagnosis of present malarial infection ; but the failure to find them by the ordinary techniques cannot justify a confident diagnosis that malaria is certainly absent.'

If, however, the figures furnished in the various tables are consulted, more especially Table X and the three cases recorded in Table VIII, it will be seen that a combination of urobilin and albumin in the urine, although no certain proof, is at least strong evidence in favour of malarial infection ; whether such evidence is strong enough to warrant immediate treatment with quinine is a matter which can only be decided by the exigencies of the case and the opinion of the physician.

As regards negative findings, the absence of urobilin (which implies the absence of urobilinogen; see remarks Table I) from the urine of a suspected malaria case appears to be of very considerable value, for not less than 87 per cent. of all true cases of malaria, whether examined in England or West Africa, showed the presence of urobilin and it has been shown (Table VIII) that urobilin is sometimes a precursor of the malaria parasites and usually persists after the latter's disappearance. It is, therefore, probably legitimate to consider that a case which has had a rise of temperature within the past forty-eight hours and does not exhibit urobilin in the urine is extremely unlikely to be suffering from malaria.

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DESCRIPTIONS OF NEW MOSQUITOS FROM SOUTH AMERICA

BY
ALWEN M. EVANS

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The material dealt with in the present paper consists largely of Culicidae collected by Dr. A. Aiken Clark on the River Amazon during his voyages to and from Manáos ; other specimens were taken or reared at Manáos by Dr. H. Wolferstan Thomas and by Dr. R. M. Gordon, and in Venezuela by Dr. M. Núñez Tovar. All of the seven species are members of the genus *Culex* and all but one belong to the sub-genus *Choeroporpa*, Dyar, the members of which are small and difficult to separate on external characters ; the seventh appears to belong to *Mochlostyrax*, Dyar and Knab, a closely allied sub-genus.

Culex (*Choeroporpa*) *innominatus*, sp.n.

MALE

Head. Occiput covered with loose, flat, whitish scales with golden reflections ; except a small median triangular area, widest behind, with narrow, curved, whitish scales ; flat scales continuous in front. Upright, forked scales intermixed with the flat ones above, yellow on anterior, black on posterior half. Palpi exceeding the proboscis by the length of the last segment and almost one-half the length of the penultimate segment. Proboscis and palpi clothed with blackish-brown scales. *Thorax.* Prothoracic lobes with yellowish setae above and black ones below. Mesonotum with integument varying from ochraceous-brown to dark olivaceous-brown ; covered with golden-brown, narrow, curved scales with brassy or bronzy reflections according to the direction in which viewed. Scales at sides of ante-scutellar space and on scutellum brassy. Pleurae with a narrow strip of flat white scales on sternopleura ; one lower mesepimeral bristle present. *Legs.* Clothed with very dark, blackish-brown scales with purplish reflections ; femora pale beneath. *Wings.* Distal half of vein I,

apical halves of forks of veins II and IV and apex of vein III with dense broad scales; lateral squames (Christophers 1923) in these positions with 7 to 9 striae and the width sometimes exceeding one-third of the length (from insertion to tip). *Abdomen.* Dorsum with narrow, basal, white bands, present on segments 2 to 7 in one specimen, on segments 3 to 7 in two specimens and on segments 4 and 5 in one specimen; in the fifth specimen the abdomen was lost, but it was recorded that bands were present on all but the first segment.

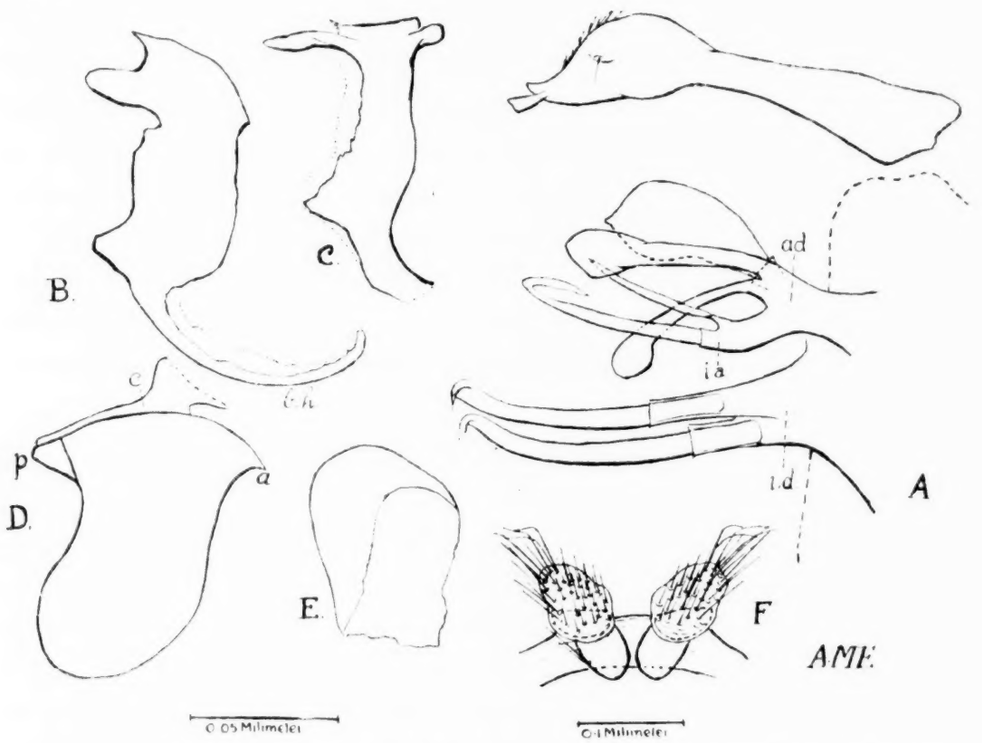

FIG. 1. *Culex* (*Choeroporpa*) *innominatus*, n.sp. Details of male hypopygium, drawn from stained preparations. A. Clasper and lobes of side-piece. *i.a.*—inner arm of outer division; *i.d.*—inner division of lobe of side-piece; *a.d.*—outer division of lobe of side-piece. B and C. Lateral plate of phallosome, lateral aspect, under different conditions of pressure. *b.b.*—basal hooks. D. Basal apodeme, flattened by pressure. *a.*—angle which articulates with parameral plate; *c.*—chitinous connection with basal arm of tenth sternite; *p.*—basal projection. E. Plate at base of side-piece. A—E to the same scale.

HYPOPYGIUM (fig. 1). The important features by which this species may be distinguished are shown in the accompanying illustration. The appendages of the outer division of the lobe of the side piece (fig. 1, A, *a.d.*) are two filaments with enlarged rounded apices and a leaf. The lateral plates of the phallosome (Christophers and Barraud, 1923) (fig. 1, B and C) even when mounted

in lateral aspect exhibit a remarkable diversity of form owing to slight differences in orientation, the shape and relative sizes of the horns or teeth appearing totally unalike in different specimens as shown by the illustrations. The basal apodeme (*ibid.*) (fig. 1, D) is subject to variation in shape according to the direction and amount of pressure put upon it. The projecting portion (*p.*) is curved at right angles to the plane of the rest of the plate, and unless the structure is completely flattened out, a greater or less degree of foreshortening results. The thin, indefinite piece of chitin (*c.*) forms a connection between the basal apodeme and a lateral projection from the base of the tenth sternite. The relative position of the ' plate at the base of the side piece ' and the basal apodeme may be seen by referring to fig. 6, C.

Wing length : *c.* 2·4 mm.

Paratypes : 2 ♂♂ taken on River Amazon, 1915, Dr. A. Aiken Clark. Co-types : 2 ♂♂ taken at Palo Negro, Venezuela, 30.VIII.22, and 1 ♂ taken at Mariara, Estada Carabobo, Venezuela, 11.VIII.22, Dr. M. Núñez, Tovar.

Culex (*Choeroporpa*) *clarki*, sp.n.

MALE

Head. Occiput entirely clothed with flat, rather closely appressed scales and a few upright, forked ones above. Flat scales appearing dark bluish-grey when viewed from above but changing to greyish white, dark sepia, or golden when the head is rotated, recalling the head scales of certain species of *Wyeomyia*. A small patch of creamy-white scales at lateral angles. Upright, forked scales consisting of a very few pale yellow ones on the anterior half, and more numerous black ones behind. Palpi exceeding the proboscis by almost the length of the last two segments. Palpi and proboscis with very dark blackish-brown scales. *Thorax.* Prothoracic lobes with long, coarse, dark brown setae. Mesonotum. Integument dark sepia, clothed with dark, bronzy, narrow, curved scales. Scutellum with integument of lobes as that of the mesonotum, but integument between the lobes tawny ; scales with brassy reflections. Pleurae with two conspicuous roundish patches of flat white scales, one on each side of the upper half of the suture separating the sterno-pleura from the mesepimeron, and an elongated patch below on

the sternopleura. One large mesepimeral bristle present. *Legs* very dark scaled. *Wings.* Apex of vein I, apical half of the forks of vein II, apex of vein III and apices of forks of vein IV, densely clothed with short broad scales, the greatest width of the lateral squames about one-third or slightly less than one-third the length, widest on apical third and with from 5 to 7 (very rarely 8) striae. *Abdomen.* Dorsum entirely clothed with dark, blackish-brown scales. Laterally, last three segments with small basal, white, triangular spots. Venter with segments 4, 5 and 6 (and possibly 7) with proximal white bands, narrow on segment 4, nearly half the width of the segment on 5 and 6.

Wing length : *c.* 2·6 mm.

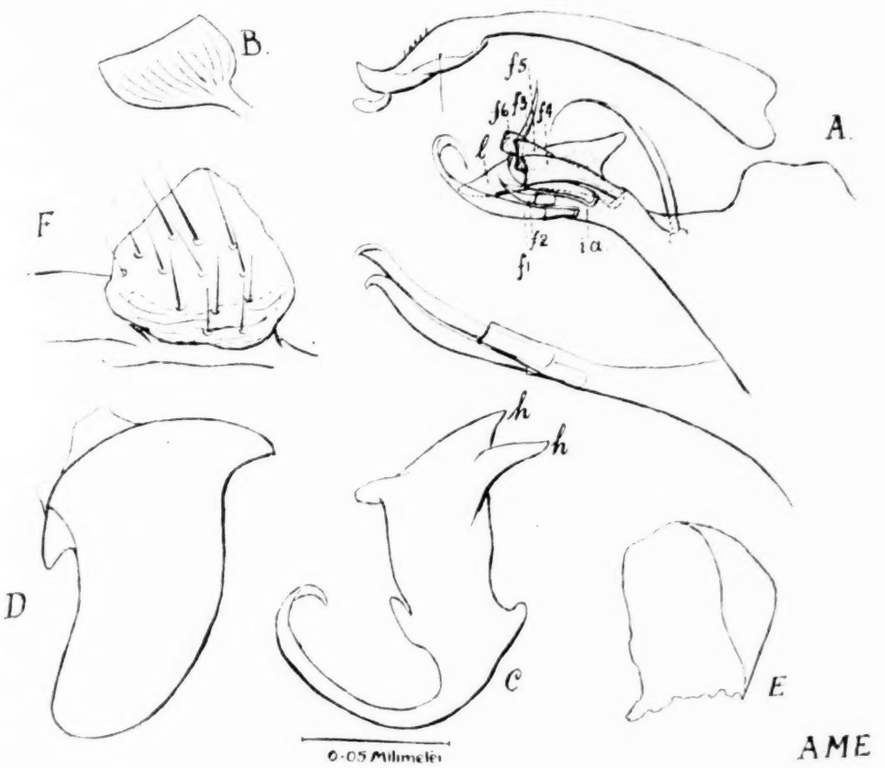

FIG. 2. *Culex* (*Choeroporpa*) *clarki*, sp.n. Details of male hypopygium, drawn from stained preparations. A. Clasper and tip of side-piece with lobes. *f.*1 to *f.*6—filaments on outer division of lobe of side-piece ; *i.a.*—inner arm of outer division of lobes ; *l.*—leaf. B. Leaf of outer division in profile. C. Lateral plate of phallosome with basal hooks, lateral aspect. *b.b.*—apical dorsal horns. D. Basal apodeme flattened by pressure. E. Plate at base of side-piece. F. One of ninth tergites.

HYPOPYGIUM (fig. 2). The enlarged distal portion of the clasper is evidently comparatively narrow, but as it appeared collapsed in all the preparations, it is possible that it is really wider than as

shown in fig. 2, A. The appendages of the outer aspect of the outer division of the lobe of the side piece are somewhat complicated. Arising next to the inner arm is a rather long pointed and curved filament (*f.*3) partly hidden (in fig. 2, A) by the three curved filaments (*f.*4, *f.*5, and *f.*6), which are superimposed one above the other ; the striated leaf (*l.*) arises from a large insertion, has the surface undulating, and projects almost at right angles to the axis of the lobe and the other appendages. In consequence of this, the true shape of the leaf cannot be seen in preparations mounted to show the filaments ; fig. 2, B, was therefore drawn from the specimen when orientated to display the leaf, before mounting in the position shown in fig. 2, A. The two dorsal horns of the phallosome are large and prominent and the small projection on the ventral side near the base seems to be a constant feature. The ninth tergites are separated by a distance slightly less than the width of one of them (fig. 2, F).

Wing length : *c.* 2·6 mm.

Paratypes: 4♂♂ taken on River Amazon, 1915, Dr. A. Aiken Clark.

Culex (*Choeroporpa*) *tovari*, sp.n.

MALE

Head. Occiput mostly covered with flat, whitish scales with pale, yellow reflections ; whitish, narrow, curved scales occupying a median triangular area, widest behind, converging to a point in front where a tuft of narrow, curved scales projects between the eyes. Palpi and proboscis with dark brown scales ; somewhat denuded proximally. Last segment of palpi wanting, penultimate segment extending beyond the proboscis by slightly less than half its own length. *Thorax.* Prothoracic lobes mostly denuded. Mesonotum shining, dark olivaceous-brown ; almost entirely denuded, but a few isolated yellowish, narrow, curved scales remaining. Scutellum tawny-brown with a few brassy, narrow, curved scales. Pleurae with a roundish patch of flat, white scales on the sternopleura above and another on the upper part of the mesepimeron, also an elongate patch before the posterior margin of the sternopleura below ; one lower mesepimeral bristle present. *Legs.* Mostly wanting ; one mid-leg complete ; dark brown scaled. *Wings.* Lateral squames on distal parts of veins II, III, and IV, markedly

narrower than in the two preceding species, the usual number of striae being only five and the length from three-and-a-half to four times the greatest width. *Abdomen.* Dorsum: segments 2 to 7 with narrow basal, whitish bands, expanding laterally. Venter with white scales at bases of segments.

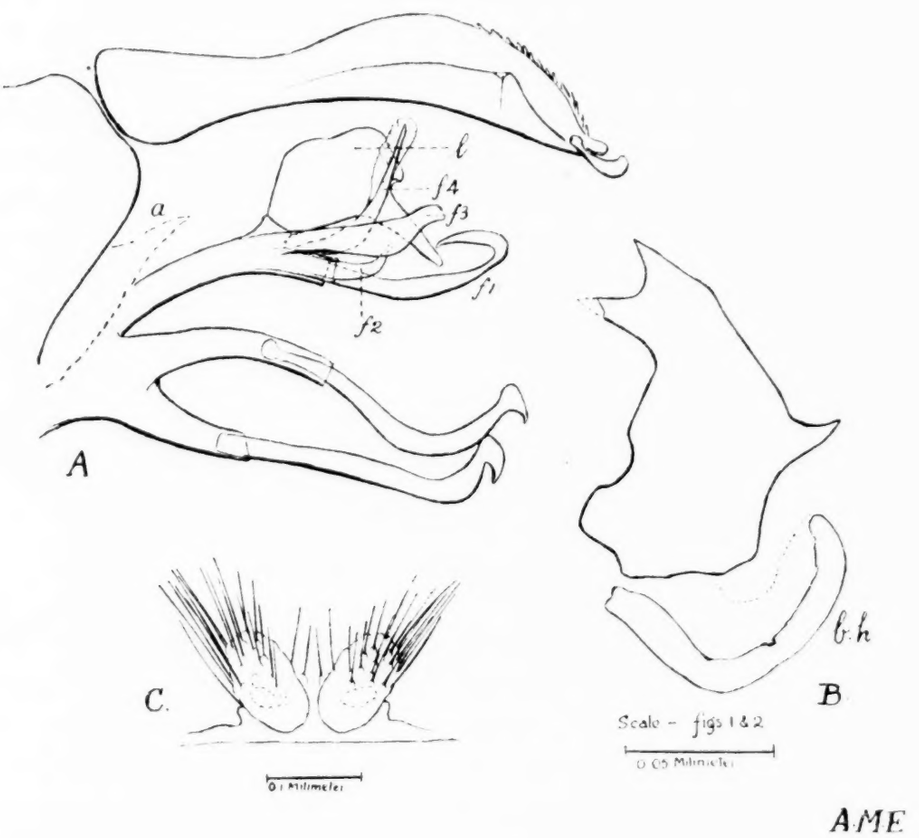

FIG. 3. *Culex* (*Choeroporpa*) *tovari*, sp.n. Details of male hypopygium, drawn from stained preparations. A. Clasper and lobes of side-piece. *a.*—artefact; *f.*1 to *f.*4—filaments of outer division of lobe of side-piece; *l.*—leaf. B. Lateral plate of phallosome, lateral aspect with basal hooks (*b.b.*) detached. C. Ninth tergites. A and B to the same scale.

HYPOPYGIUM (fig. 3). The leaf on the outer aspect of the outer lobe of the side piece (fig. 3, A), is markedly asymmetrical and undulating; distally the margin is curled so that the outline changes considerably with slight changes in the orientation. A marked feature is the striated membranous expansion surrounding the apical part of the filament (*f.*4). The short filament (*f.*2) is closely associated, if not fused, basally with the long filament (*f.*1)

so that under a low magnification it appears to be a small basal branch of the latter. The basal apodemes are not well displayed in the slide, they appear to be rather wider distally than in the preceding species (fig. 2, D). The plate at the base of the side piece resembles that of *C. manaosensis* (fig. 5, D).

Wing length : *c.* 2·8 mm.

Type: one ♂ from Palo Negro, Venezuela, 30.VIII.22, Dr. M. Núñez Tovar.

Culex (*Choeroporpa*) *gordoni*, sp.n.

MALE

Head. Occiput clothed with flat, whitish scales with pink and brassy reflections ; the numerous upright, forked scales, yellow in front, black behind. One palp present measuring 1·6 mm., the last two segments equal in length ; scales brown. Proboscis incomplete. *Thorax.* Prothoracic lobes with blackish integument. Mesonotum with integument very dark blackish brown (dark olivaceous at sides with magnification of about forty times) ; clothed with narrow, curved, bronzy scales ; bristles long, blackish. Scutellum olivaceous brown, with dull greyish-yellow scales. Pleurae very dark blackish grey ; one lower mesepimeral bristle present. *Legs.* Dark brown scaled. *Wings* with the lateral squames on the apical parts of veins II, III and IV, rather broad and dense ; widest type of scales in these positions with eight striae, width sometimes more than one-third of the length. *Abdomen.* Scales of dorsum brown with coppery reflections, segments 4, 5, 6 and 7 with small lateral, triangular, white spots.

HYPOPYGIUM (fig. 4). The most characteristic feature is the greatly expanded apex of the phallosome plate (fig. 4, B) which is furnished with a double row of teeth along its distal edge. The phallosome is very closely associated with the parameral plates (not shown in the figure). The outer division of the lobe of the side piece is furnished on its outer aspect with four filaments : *f.*1, *f.*2, *f.*3 and *f.*4, the last named being almost obscured (in the figure) by the others. The basal apodeme is very similar to that of *C. clarki* (fig. 2, D). Tenth sternites with about twelve very delicate teeth.

Wing length : *c.* 2·1 mm.

Type: ♂ bred from a pool at the Bosque, Manáos, 29.XII.21, Dr. R. M. Gordon.

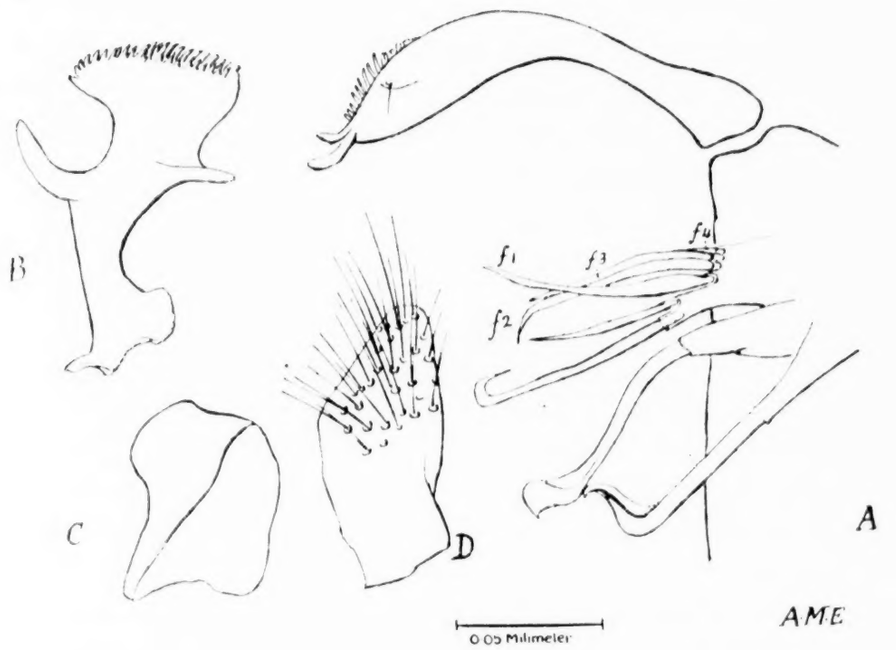

FIG. 4. *Culex* (*Choeroporpa*) *gordoni*, sp.n. Details of male hypopygium, drawn from unstained preparations. A. Apex of side-piece with clasper and lobes. *f.* 1 to *f.* 4—filaments on outer aspect. B. Lateral plate of phallosome, lateral aspect, basal hooks omitted. C. Plate at base of side-piece. D. One of the ninth tergites.

This species evidently comes near to *C.* (*Choeroporpa*) *educator*, Dyar and Knab, but I have hesitated to identify it with that species because, although the description of the mesosome (Dyar, 1920) might possibly apply to the Manáos specimen, if Dyar's figure (1918, Pl. IV, fig. 17) represents this structure in lateral view, the two species are clearly distinct.

Culex (*Choeroporpa*) *manaosensis*, sp.n.

MALE

Head. Occiput covered with flat whitish scales with metallic reflections except a triangular area of narrow, curved, whitish scales converging to a median tuft projecting between the eyes; upright, forked scales numerous, black. Palpi and proboscis covered with very dark, blackish-brown scales. Last segment of each palp missing, but penultimate segment extending beyond the proboscis by about one-half of its own length. Long hairs of antennae blackish-brown. *Thorax.* Prothoracic lobes very dark, with black bristles. Mesonotum with integument shining blackish-

brown ; scales bronzy ; that of scutellum and ante-scutellar space olivaceous-brown. Pleurae olivaceous-brown, a row of flat white scales at posterior border of sternopleura ; bristles black, rather fine, one lower mesepimeral bristle. *Legs.* Fore and mid legs with blackish scales, hind legs missing. *Wings* with widest lateral squames on veins II, III and IV mostly with seven, rarely with eight striae. *Abdomen.* Dorsum with blackish-brown scales ; all but first three segments with small, triangular, lateral, whitish spots. Venter with basal, whitish bands.

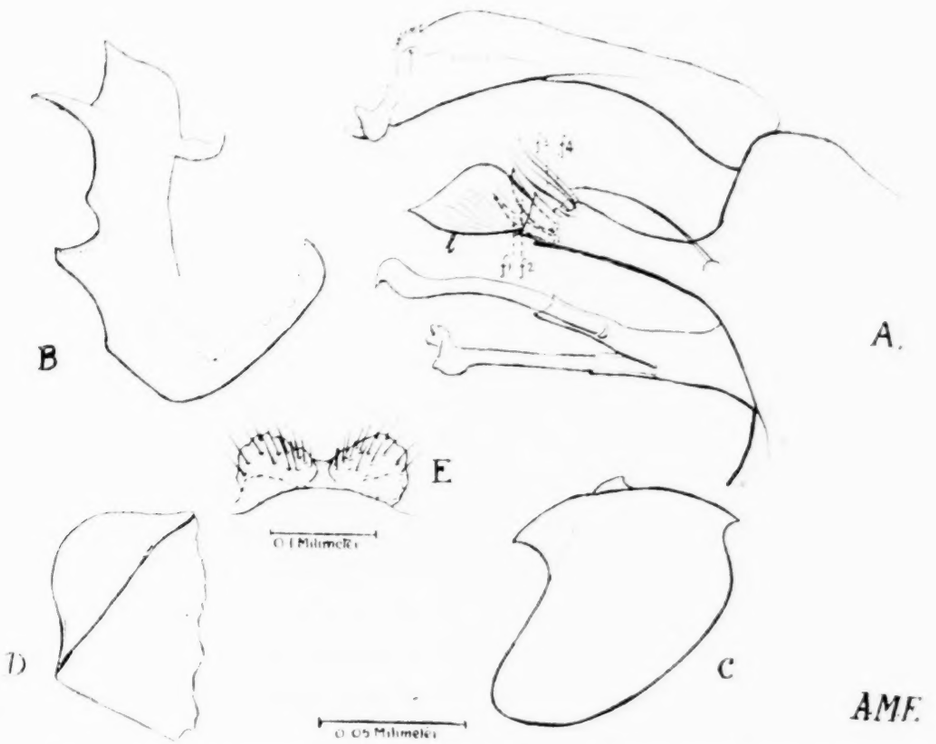

FIG. 5. *Culex* (*Choeroporpa*) *manaosensis*, sp.n. Details of male hypopygium, drawn from stained preparations. A. Apex of side-piece with clasper and lobes ; *f.* 1 to *f.* 4—filaments of outer division ; *l.*—leaf. B. Lateral plate of phallosome with basal hooks, lateral aspect. C. Basal apodeme, flattened by pressure. D. Plate at base of side-piece. E. Ninth tergites. A—D to the same scale.

HYPOPYGIUM (fig. 5). The leaf borne by the outer division of the lobe of the side piece (fig. 5, A, *l.*) is stiff and erect with a very prominent insertion ; in the figure it obscures two of the four short, pointed filaments (*f.*1 and *f.*2) which are indicated by dotted lines. The tenth sternites have about twelve teeth.

Wing length : *c.* 2·5 mm.

Type : one ♂ taken on the Wharf, Manáos, 5.XII.23, Dr. A. Aiken Clark.

Culex (*Choeroporpa*) *thomasi*, sp.n.

MALE

Head. Occiput covered with flat, whitish scales with yellow reflections; except a median triangular area, widest behind, converging to a point in front occupied by whitish, narrow, curved scales, a tuft of narrow, curved scales projecting between the eyes. Upright, forked scales yellow in front, black behind. Palpi and proboscis covered with rather light, brown scales; palpi exceeding proboscis by the length of the last segment and half the preceding one. *Thorax.* Prothoracic lobes with blackish bristles. Mesonotum with ochraceous-brown integument and golden, narrow, curved scales. Scutellar scales brassy, bristles brown with golden reflections. Pleurae purplish-brown, light tawny around sutures, partly denuded, a row of flat, white scales along the posterior border of the sternopleura; one lower mesepimeral bristle. *Legs* unbanded, clothed with brown scales with coppery reflections. *Wings* with the widest lateral squames on veins II, III and IV with seven striae and the greatest width in many cases more than one-third of the length. *Abdomen.* Dorsum with dark brown scales and on segments 2 to 6, well defined, whitish, basal bands, narrow on second segment; segments 7 and 8 with irregular, basal, whitish bands.

HYPOPYGIUM (fig. 6). The claspers have not been figured, as the distal portions are collapsed in the preparation. In a sketch made before mounting, the distal third is rather abruptly widened, the eye-like spine and the sub-apical appendage are present, and the narrow terminal portion of the clasper is upturned as in most of the species here dealt with.

Wing length: *c.* 2·2 mm.

Type: one ♂ bearing the data 'Swamp water from Amatory, Manáos, Dr. H. Wolferstan Thomas,' 1910.

This specimen was determined on external characters as *Culex* (*Melanoconion*) *humilis*, Theo., but as the hypopygial characters showed that it was not a *Melanoconion*, the determination seemed open to doubt. Mr. F. W. Edwards kindly compared the external characters with the type of which he says 'only the head and wing are much use for comparison.' Mr. Edwards mentioned certain differences between the two specimens, and in view of these differences, and the condition of the type specimen, it seems safer

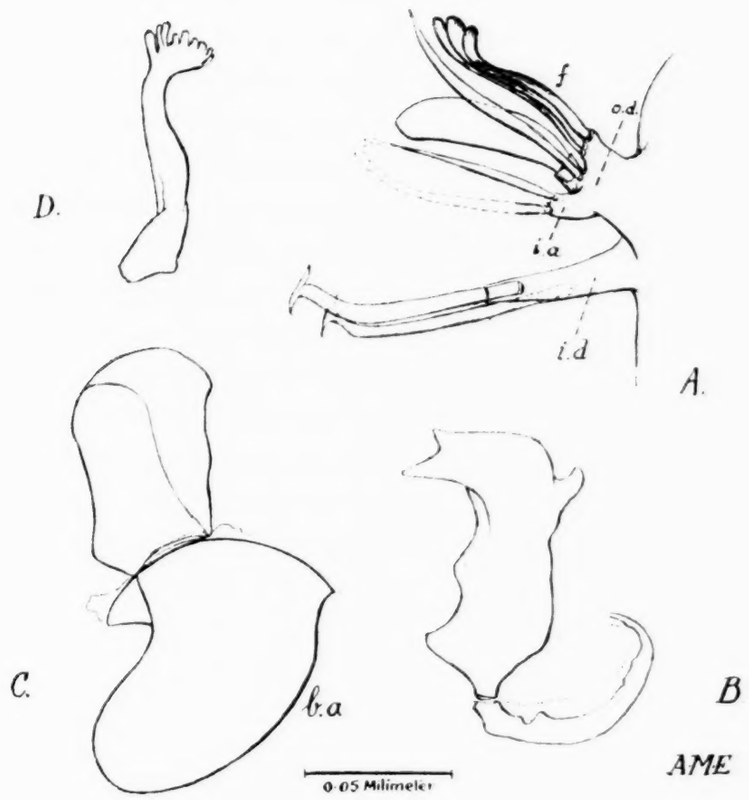

FIG. 6. *Culex* (*Choeroporpa*) *thomasi*, sp.n. Details of male hypopygium, drawn from stained preparations. A. Lobes of side-piece. *i.a.*—inner arm of outer division; *i.d.*—inner division; *f.*—group of three filaments; *o.d.*—outer division. B. Lateral plate of phallosome with basal hooks, lateral aspect. C. Basal apodeme (*b.a.*) and plate at base of side-piece. D. Tenth sternite.

to regard the specimen from Manáos as a distinct species. The fact that the type was taken at San Paulo is an additional reason for adopting this course.

Another specimen determined as *M. humilis*, Theo. was a true *Melanoconion*, which I have identified with *C.* (*Melanoconion*) *dunni*, Dyar. Mr. Edwards found that it was specifically distinct from the type of *M. humilis*, Theo.

Culex innovator, sp.n.

MALE

Head. Occiput mostly covered with flat, whitish scales, with pale yellowish and pink, metallic reflections, appearing bluish grey in certain lights. A broad, median patch of narrow, curved, whitish scales behind extending less than half-way to the anterior margin.

Upright, forked scales all dark. Palpi and proboscis brown scaled. Palpi exceeding the proboscis by nearly the length of the last two segments. *Thorax.* Prothoracic lobes tawny with brown bristles. Mesonotum with integument bright ochraceous-brown, covered with very small, dark-brown, narrow, curved scales with golden reflections. Scutellum tawny, scales with brassy reflections. Pleurae pale fawn-coloured ; an elongate patch of flat, white scales along the posterior border of the sternopleura, one lower mesepimeral bristle. *Legs* dark-brown scaled. *Wings* with widest lateral squames not as broad as in *C. innominatus*, those on veins II, III and IV not exceeding six striae and having the greatest width less than one-third of their length. *Abdomen* dark-brown scaled above, segments 4, 5 and 6, with small, basal, lateral, triangular, pale spots, segment 7 with basal, lateral, pale spots tending to form an irregular band, segment 8 variable.

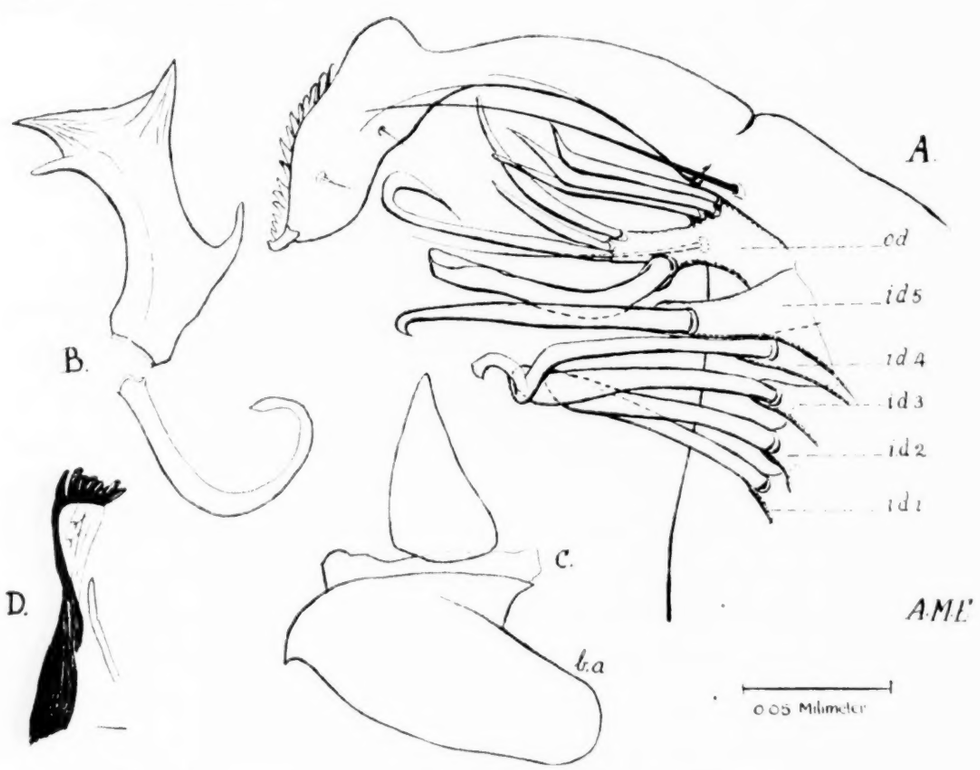

FIG. 7. *Culex innovator*, sp.n. Details of male hypopygium, drawn from stained preparations. A. Apex of side-piece with clasper and lobes. *i.d.* 1 to *i.d.* 5—inner divisions of lobe of side-piece ; *o.d.*—outer division. B. Lateral plate of phallosome with basal hooks, lateral aspect. C. Basal apodeme and plate at base of side-piece. D. Tenth sternite.

HYPOPYGIUM (fig. 7). The most marked feature is the greatly sub-divided inner division of the lobe of the side-piece. It consists

of five distinct divisions (*i.d.* 1 to *i.d.* 5) arranged obliquely to the axis of the side-piece. The three innermost divisions (*i.d.* 1 to *i.d.* 3) bear at their extremities long, flattened filaments gradually widening distally and obliquely rounded. The extremities of these filaments lie in close proximity, so that it is difficult to display them separately in mounting them. The fourth division (*i.d.* 4) alone bears a filament or rod similar to those borne on the inner division of the side-piece in species of *Choeroporpa* (figs. 1 to 6, A). The striated appearance of the chitin forming the teeth of the plates of the phallosome (fig. 7, B) appears to be a constant feature.

Wing length : *c.* 2·4 mm.

Paratypes : three ♂♂ from the River Amazon, 1915, Dr. A. Aiken Clark.

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NOTES ON SOME AFRICAN CERATOPOGONINAE—SPECIES OF THE GENUS *LASIOHELEA*

BY

A. INGRAM

AND

J. W. S. MACFIE

(*Received for publication* 8 *September*, 1924)

PLATE XXII

In the collection of Ceratopogonine midges made by us in West Africa, mainly between the years 1919 and 1922, were a number of specimens (including one male) which appear to us to belong to the genus *Lasiohelea*. In examining these specimens we have had the advantage of comparing them with named examples of *L. stylifer* presented by Dr. Lutz to the Liverpool School of Tropical Medicine, and with the co-types of *L. lefanui* (Carter). The majority of our specimens are undoubtedly *L. lefanui*, but others appear to be new species and are here briefly described.

It is extremely difficult to define the genus *Lasiohelea*, or to state precisely in what respects it differs from the genera *Forcipomyia* and *Atrichopogon*. Kieffer who erected the genus in 1921 for his species *Atrichopogon pilosipennis*, gives only the following definition : hairiness of the wings as in *Forcipomyia* ; empodium as long as the claws, with short hairs ; palps having the last segment long ; otherwise as in *Atrichopogon*. We have not had an opportunity of examining *L. pilosipennis*, but we have examined *L. stylifer* which is generally admitted to belong to the same genus, and as regards this species at any rate, Kieffer's definition is scarcely adequate, for in it the hairiness of the wings is less dense than in *Forcipomyia* and of a somewhat different character, the hairs on the empodium are not

short, the last segment of the palp is scarcely, if at all, longer than the penultimate segment, and in other respects (*e.g.*, the size of the microtrichia and the form of the radial cells of the wing) there are marked differences between it and *Atrichopogon*.

More recently (1922) Edwards has published an interesting note on the genus, in which he refers to its kinship with *Atrichopogon* and *Forcipomyia* more specifically than does Kieffer. *Lasiohelea* resembles *Atrichopogon*, he says, in the structure of the antennae and in wing venation, having a very long second radial cell (which however is narrower than in *Atrichopogon*) and an obliterated first radial cell ; it resembles *Forcipomyia* in having the wings rather densely clothed with macrotrichia, but the hairs are less close-lying than in *Forcipomyia* and there are bare lines adjoining the veins, as in *Atrichopogon*. He states also that the microtrichia on the wings of *Lasiohelea* are ' smaller than those of *Atrichopogon*, but more obvious than those of *Forcipomyia*.'

We are doubtful if there is any real difference between the microtrichia of *Lasiohelea* and those of *Forcipomyia* ; in both genera they are small and slightly variable in size in different species ; but between the microtrichia of *Lasiohelea* and those of *Atrichopogon* the difference is much more marked and may serve as a ready means of separating these genera. It is more difficult to find a clear distinction between *Lasiohelea* and *Forcipomyia*. The hairiness of the wings is a variable character and the bare areas along the veins are not always present in *Lasiohelea* ; the structure of the antennae is also variable within wide limits, so that for diagnostic purposes we are apparently forced to rely on the form of the radial cells of the wing, the first cell in *Lasiohelea* being obliterated and the second long and narrow, extending beyond the middle of the wing. These cannot be considered satisfactory characteristics, for the first radial cell is not always entirely obliterated in *Lasiohelea* and *Forcipomyia* (as can readily be demonstrated by appropriate staining) and the second radial cell varies considerably in length and width in *Forcipomyia* and sometimes extends beyond the middle of the wing. The genera in which the thorax is not prolonged over the head as a hood, the empodium well developed, as long as the claws and with long hairs, and the femora and fifth tarsal segments unarmed, may perhaps, however, be distinguished as follows :—

1. Costa reaching beyond the middle of the wing, usually two-thirds its length ; second radial cell usually very long ; fringe on the posterior border of the wing composed of simple hairs.....2

   Costa usually not reaching beyond the middle of the wing or only slightly ; second radial cell not very long ; fringe on the posterior border of the wing with hairs which are usually lanceolate and sometimes pubescent or sub-plumose.....3

2. First and third veins of the wing forming two distinct cells ; microtrichia large and conspicuous ; fringe on the posterior border of the wing composed of a single row of alternating long and short, simple, straight hairs..... *Atrichopogon*

   First radial cell obliterated or nearly, second very narrow ; microtrichia minute ; fringe on the posterior border of the wing composed of long hairs between two rows of shorter, oblique, hairs..... *Lasiohelea*

3. Wings densely clothed with hairs or scales.....4

   Wings with microtrichia only. (Fifth segment of palp sub-spherical, fourth long ; costa not reaching beyond the middle of the wing ; first tarsal segment of hind legs half the length of the second)..... *Microhelea*

4. Scales on wings and legs..... [*Lepidohelea*]

   Without scales..... *Forcipomyia*

So far as we can determine from the examination of our specimens collected in West Africa, and of the examples of *L. stylifer* and *L. lefanui* in the collection of the Liverpool School of Tropical Medicine, the following are the chief characters of the genus *Lasiohelea*.

LASIOHELEA, Keiffer, 1921

Head. Eyes bare or more or less hairy ; broadly contiguous above, but with the facets sometimes rather widely separated. Palpi sometimes with the fifth segment longer than the fourth ; third segment with or without a sensory pit. Mouth parts well chitinised. Labium fleshy, somewhat similar to that of *Culicoides*. In *L. lefanui* the labrum tapers distally, ends in a sharp point, and is not armed with teeth ; the mandibles are strongly chitinised, taper in the distal third, end in sharp points, and bear a row of

about twenty-five small teeth along the distal margin; the maxillae are shorter than the mandibles and less highly chitinised, and bear fewer teeth, about fifteen, distally; and the hypopharynx is strongly chitinised, tapers distally, and ends sharply. *Antennae* of female with flagellum segments sometimes forming an almost continuous series, sometimes with the basal segments short and broad and the last five segments much elongated, an abrupt change of shape taking place between the tenth and eleventh segments. In none of the species examined by us were the basal segments so short and broad (' transversaux ') as they often are in *Atrichopogon*. Terminal segments of antenna not definitely sculptured, but in some species irregularly chitinised and having in consequence a patterned appearance. Whorls of hairs on the basal segments composed of about eight to twelve hairs; large spines usually only a little stouter than the hairs, about as long as the segments, straight or nearly, and tapering to more or less pointed extremities. *Thorax*. Scutellum bearing few bristles and hairs, the former in a transverse row, often with the most lateral bristle on each side somewhat apart from the rest. *Wings* usually short and broad, with a rounded distal extremity. Fringe on the posterior part of the wing composed of long hairs between two rows of shorter oblique hairs, not as in *Atrichopogon* of practically a single row of alternating long and short straight hairs. Fringe hairs apparently simple and straight. Border of wing thickened, especially anteriorly; the gap just beyond the end of the costa distinct. Microtrichia small, as in *Forcipomyia*, smaller than in *Atrichopogon*. Macrotrichia covering the wings more or less completely and densely, but not so densely as in *Forcipomyia*, slightly curved, slender, hair-like, tapering to very sharply pointed ends. Those on the costa and the anterior veins sometimes broader, scale-like. There may or may not be definite bare areas along the veins, as in *Atrichopogon*. Costa reaching well beyond the middle of the wing, usually two-thirds the length. Sub-costa strongly developed, bearing stout hairs or scale-like hairs. First radial cell usually obliterated and at most very narrow and slit-like. Second radial cell very long and usually very narrow. Cross-vein oblique. Petiole of the fourth vein short, almost obsolete, ·.mi obsolete proximally and sometimes very indistinct for r entire lengths. Bifurcation of the fifth vein

proximal to the level of the end of the costa, the rami terminating at the wing margin, the one proximal and the other distal to this level. *Legs.* First tarsal segment longer than the second on all the legs ; on the hind legs usually two and a half to three times as long, but in one species examined by us, shorter, a little less than twice the length of the second. Terminal tarsal segments sub-cylindrical. In some species the tarsal segments bear fringed, striated scales in addition to hairs. Claws short, equal, usually rather stout and having a thickening about the middle. Empodium nearly as long as the claws, hairy, the hairs actually long, but curved and thus in some views appearing short. *Abdomen.* Well clothed with hairs and often with a small admedian tuft on each side of the eighth sternite. There may be either a single spermatheca or two spermathecae. The hypopygium of the single male examined by us resembled in some respects that of *Forcipomyia ingrami.*

KEY TO THE WEST AFRICAN SPECIES OF THE GENUS *LASIOHELEA.*

1.	With scales on the legs.....2	
	Without scales.....5	
2.	Hind legs with first tarsal segment less than twice the length of the second.....	*L. brevitarsata*, sp.n.
	Hind legs with first tarsal segment two to three times the length of the second.....3	
3.	Third segment of palp without a sensory pit.....	*L. nigeriae*, sp.n.
	Third segment of palp with a sensory pit.....4	
4.	Spermatheca single, sub-spherical, the basal portion not chitinised.....	*L. lefanui* (Carter) var. *squamipes*,* var. n.
	Spermathecae two, pyriform.....	*L. caliginosa*, sp.n.
5.	Eyes hairy.....	*L. litoraurea*, sp.n.
	Eyes bare.....6	
6.	Antenna with the segments of the flagellum forming an almost continuous series, with no abrupt change of shape between the tenth and eleventh segments	*L. inconspicuosa*, sp.n.
	Antenna with the last five segments much elongated, with an abrupt change of shape between the tenth and eleventh segments.....	*L. lefanui* (Cart.)

* This East African species is included here because it is very closely allied to *L. lefanui* and may eventually be found to occur also in West Africa.

LASIOHELEA NIGERIAE, sp.n.

Length of body* (two females and one male), 1·3 mm. to 1·4 mm.; length of wing, 0·9 mm. to 1·1 mm.; greatest breadth of wing, 0·3 mm. to 0·35 mm. The male is slightly longer than the females, and its wings slightly longer and narrower.

A dark brown insect with yellowish legs, closely resembling *L. lefanui* (Carter), but differing from the three co-types of this species in the following respects. *Head.* Eyes bare above, slightly hairy

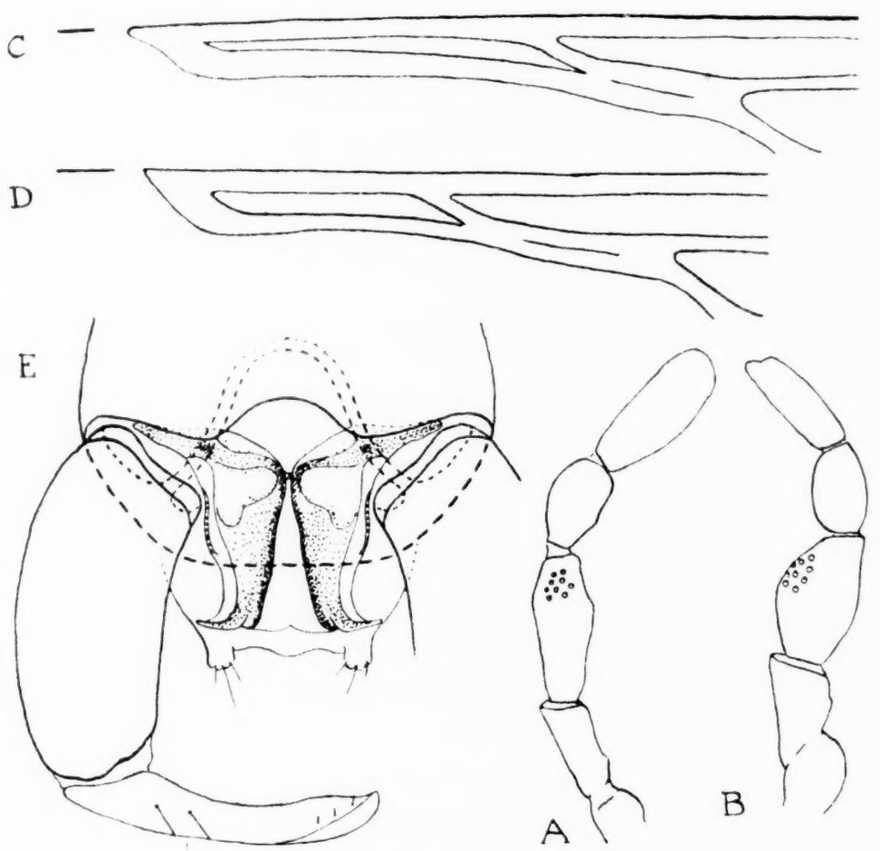

FIG. 1. *Lasiohelea nigeriae*, sp.n.: A. palp, ♂; B. palp, ♀; C. radial cells, ♀; D. radial cells, ♂; E. hypopygium, ♂, ventral view. [C. and D. × c. 265, others × c. 400.]

below and at the inner margins; broadly contiguous above in both sexes. Palpi (fig. 1, A and B) in both sexes with the fifth segment longer than the fourth; in the female the third segment a little inflated in the middle, in the male hardly at all, in both sexes without a sensory

* In all cases this measurement is taken from the anterior margin of the thorax to the tip of the abdomen of specimens mounted in pure carbolic acid.

pit but bearing sensory hairs on the inner anterior aspect. *Antennae.* In the female segments 4 to 10 similar to those of *L. lefanui*, the length and breadth of segments 4 and 10 in one specimen measuring 6 by 8, and 9 by 6 units* respectively ; segments 11 to 15 elongated, rather longer than in *F. lefanui*, the lengths of the eleventh and fifteenth segments in one specimen measuring 20 and 25 units respectively. The combined length of segments 11 to 15 greater than that of segments 3 to 10 and twice as great as that of segments 4 to 10, the three actual measurements in one specimen being 109, 61, and 51 units respectively. In the single male in our possession the antennae are damaged, the terminal segments missing. Torus very large and very dark brown ; flagellum segments brown. Segments 4 to 11 all about the same length, 12 units, but rapidly diminishing in greatest breadth from a little over 10 units in the case of the fourth segment to a little over 5 units in that of the eleventh. *Thorax.* Scutellum with fewer small hairs, about six in the female and one or two in the male. *Wings* (Pl. XXII, fig. 1) similar to those of *L. lefanui* but with the hairs on the costa and the anterior veins dark brown, lanceolate, scale-like. Bare areas along the veins well marked. Wings not so densely clothed with macrotrichia as in *L. lefanui*. At the base of the wing, between the fourth and fifth veins, one or two rows of macrotrichia ; at the level of the bifurcation of the fifth vein, two rows between the fourth and fifth veins, three rows between the rami of the fourth vein, and two rows between the third and fourth veins. Microtrichia very minute. The tip of the wing rounded but not as broad as the broadest part of the wing. In the male the wings are slightly longer and narrower than in the female, costa reaching nearly to two-thirds of the length of the wing from the base (35 : 58), first radial cell obsolete, second long and narrow but neither so long nor so narrow as in the female (fig. 1, C and D), bifurcation of the fifth vein a little proximal to the end of the costa. *Legs.* Tarsal segments somewhat infuscated, bearing numerous striated scales which are most conspicuous on the distal segments. First tarsal segment of all the legs, over twice the length of the second, the actual measurements of the first two tarsal segments of the fore, middle, and hind legs of one female being 50 and 21, 47 and 22, and 59 and 26 units respectively.

* The unit referred to is 3·7*μ*.

Abdomen bearing lanceolate, scale-like hairs similar to those on the wing, in addition to ordinary hairs. Abdomen of the male rather dark brown, narrow, with a conspicuous, dark brown hypopygium. Spermatheca single, sub-spherical, of the same form as that of *L. lefanui*, diameter about 60μ, the base not chitinised ; the diameter at the level where the chitinisation ceases about 25μ.

HYPOPYGIUM (fig. 1, E). *Ninth segment* dark brown : tergite hairy, with a posterior extension similar to that of *Forcipomyia* ; sternite with fairly numerous hairs restricted to the anterior part, slightly notched in the middle line posteriorly. *Forceps* well developed : side-pieces about twice as long as broad, moderately hairy, their proximal ends with a highly chitinised rim ; claspers brown, well chitinised, their ends somewhat spoon-shaped. *Harpes* : there are no posteriorly projecting plates. The strongly chitinised dorsal root-like processes arising at the bases of the side-pieces are long and join anteriorly across the middle line forming a wide arch somewhat similar to that of *Forcipomyia ingrami*. *Aedoeagus* a highly complicated structure, partly chitinised, partly membranous ; in ventral view the chitinised portions appear as two roughly triangular objects with their apices apposed in the middle line a little posterior to the margin of the ninth sternite. In lateral view there is seen to be on each side at the distal end a process directed dorsally.

NIGERIA : Calabar, February, 1922, 1 ♂, 2 ♀♀ (Dr. E. C. Braithwaite).

Four specimens (♀♀) of *L. stylifer*, named by Dr. Lutz, in the collection of the Liverpool School of Tropical Medicine show scales similar to those of the species described above ; but on the three co-types (♀♀) of *L. lefanui* in the same collection, and on some specimens collected by us in Nigeria and the Gold Coast, and on others sent to us by Dr. J. R. C. Stephens from Ilorin, Nigeria, which appear to us to be *L. lefanui*, we are unable to find any such scales. The species described above, therefore, resembles *L. stylifer* even more closely than *L. lefanui*, but there are certain differences, *e.g.*, in the palps which in *L. stylifer* have a large sensory pit in the third segment and the fourth and fifth segments subequal, and in the absence of a comparison of the genitalia of the male, we think the two species must be regarded as distinct although undoubtedly closely allied.

LASIOHELEA BREVITARSATA, sp.n.

Length of body (one female), 0·9 mm. ; length of wing, 0·8 mm. ; greatest breadth of wing, 0·3 mm. Resembling the preceding species, *L. nigeriae*, in almost every respect, but smaller and with relatively shorter first tarsal segments.

Head. Eyes very slightly hairy, as in *L. nigeriae*. Palpi as in *L. nigeriae*. *Antennae* similar to those of *L. nigeriae* but smaller. Segments 4 and 10 measuring approximately 6 by 6, and 7·5 by 5 units respectively. Segments 11 to 14 sub-equal, about 15 by 5 units ; the fifteenth segment a little longer, about 20 by 5 units, and ending in a blunt stylet. The combined length of segments 11 to 15 (78 units) greater than that of segments 3 to 10 (50 units), and not quite twice that of segments 4 to 10 (43 units). *Thorax.* Scutellum bearing a transverse row of eight bristles and a few (six) smaller hairs as in *L. nigeriae*. *Wings* (Pl. XXII, fig. 2). Shape of wing and distribution of macrotrichia as in *L. nigeriae*. Costa reaching nearly two-thirds of the wing length (25 : 43). Halteres somewhat infuscated. *Legs* bearing scales as in *L. nigeriae*. First tarsal segment of the fore and middle legs about twice, and of the hind legs a little less than twice the length of the second ; the actual measurements of the first and second tarsal segments of the fore, middle, and hind legs being 37 and 18, 35 and 17, and 43 and 24 units respectively. *Abdomen* as in *L. nigeriae*. Spermatheca single, sub-spherical, of the same form as in *L. lefanui*, diameter about 50μ, the extreme base not chitinised, the diameter at the level where the chitinisation ends, about 15μ.

Gold Coast : Accra, 1920, 2 ♀♀, taken in the laboratory.

LASIOHELEA LEFANUI, (Carter) var. *SQUAMIPES*, var. n.

In a former paper (1923) we stated that we had been unable to detect any differences by which *L. stylifer* could be distinguished from the African species which we described as *L. lefanui*. In connection with the present work we have had occasion to re-examine the co-types of *L. lefanui* and specimens of *L. stylifer* named by Dr. Lutz. Whilst agreeing in most respects these specimens differ slightly in a number of minor points which may be of less than specific importance, and more notably in that whereas *L. stylifer*

has numerous scales on the legs, the co-types of *L. lefanui* appear to be entirely without scales.

In a small collection of African Ceratopogoninae kindly lent to us for examination by Dr. G. A. K. Marshall of the Imperial Bureau of Entomology, were a number of specimens of *Lasiohelea*. In one tube, to which unfortunately no label indicating locality was attached, but which probably came from Zanzibar (Dr. W. Mansfield Aders), were several specimens of a species to which brief reference must be made. These insects resemble *L. lefanui* in almost every respect, but on the legs there are a few narrow scales (not so many, however, as in the specimens of *L. stylifer* we have examined). On the wings the bare areas along the veins are distinct. At the base of the wing, between the fourth and fifth veins, are two or three rows of macrotrichia ; and at the level of the bifurcation of the fifth vein, about four rows between the fourth and fifth veins, three or four between the rami of the fourth vein, and two or three between the third and fourth veins. The distribution of macrotrichia is thus much as in *L. lefanui*, but more sparse than in *L. stylifer*, in which, in the examples examined by us, there are six or seven rows at the base between the fourth and fifth veins, and at the level of the bifurcation of the fifth vein about five rows between the fourth and fifth veins, four or five between the rami of the fourth vein, and three or four between the third and fourth veins. They differ from the species we have described above as *L. nigeriae* in having the wings more hairy, and the palps with the third segment furnished with a large but shallow sensory pit, and the last two segments sub-equal.

In the present state of knowledge of the genus, and in view of the apparent great variability of *L. lefanui*, we think it best to regard these specimens as a variety of that species and propose for them the name *Lasiohelea lefanui* (Carter) var. *squamipes*.

LASIOHELEA CALIGINOSA, sp.n.

Length of body (one female), 1·2 mm. ; length of wing, 1·0 mm. ; greatest breadth of wing, 0·4 mm. A very dark brown midge with dark brown legs on the tarsal segments of which are striated scales ; closely resembling *L. nigeriae*.

Head very dark brown. Eyes bare ; broadly contiguous above, the facets narrowly separated. Clypeus, proboscis, and palpi dark brown. Mandibles apparently not armed with teeth. Palpi (fig.2, A) with fifth segment only slightly longer than the fourth, third slightly inflated in the middle and furnished with a small sensory pit. *Antennae.* Torus very dark brown, sub-spherical, not hollowed out anteriorly, bearing several longish hairs. Flagellum segments dark brown, but not so dark as the torus. Basal segments of the flagellum bearing large spines which are a little stouter than the hairs, slightly curved, about as long as the segments, tapering to not very sharp points ; and whorls of about ten to twelve hairs. Third segment larger than the fourth, with a quite short stalk. Segments 4 to 10 short and broad, from a little broader than long to a little

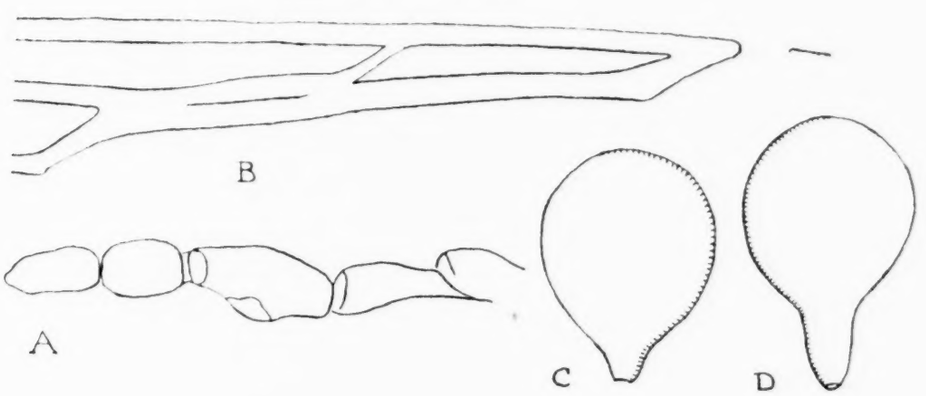

FIG. 2. *Lasiobelea caliginosa*, sp.n., ♀ : A. palp ; B. radial cells ; C. and D. spermathecae. [B. × c. 265, others × c. 400.]

longer than broad, the fourth and the tenth segments actually measuring in length and breadth 7 by 8, and 8 by 7 units respectively. Segments 11 to 15 elongated, three to nearly four times as long as broad, their lengths in one female 17, 20, 21, 22, and 27 units respectively, the terminal segment ending in a blunt sessile stylet. The combined length of segments 11 to 15 greater than that of segments 3 to 10 and about twice as great as that of segments 4 to 10, namely 107, 59, and 50 units respectively. *Thorax.* Dorsum uniformly very dark brown ; pleura dark brown. Scutellum dark brown, but not so dark as the dorsum ; bearing a transverse row of about eight bristles, and about fifteen smaller hairs. Post-scutellum

very dark brown. *Wings* (Pl. XXII, fig. 3) unadorned, but the anterior border appears darker than the rest of the wing on account of the density of the hairs in this region. Microtrichia minute. Macrotrichia covering almost the entire wing, moderately densely. Bare areas along the veins indistinct. At the base of the wing between the fourth and fifth veins three or four rows of macrotrichia ; at the level of the bifurcation of the fifth vein, about five rows between the fourth and fifth veins, about five rows between the rami of the fourth vein, and three or four rows between the third and fourth veins. The tip of the wing rounded but not the broadest part of the wing. Macrotrichia on the costa and anterior veins as in *L. nigeriae*, but narrower. Costa reaching beyond the middle of the wing (35 : 55). First radial cell obliterated, second long and narrow but not so long as in *L. nigeriae* (fig. 2, B). Bifurcation of the fifth vein well proximal to the end of the costa. Halteres with brown knobs. *Legs* rather dark brown, the tarsal segments paler brown than the proximal segments ; well clothed with shortish hairs, and bearing striated scales on the tarsi. First tarsal segment over two-and-a-half times as long as the second on all the legs, *i.e.*, measuring in one specimen 53 : 19, 51 : 18, and 63 : 22 on the fore, middle, and hind legs respectively. Claws stout, equal, about half the length of the fifth tarsal segment. Empodium long and hairy. *Abdomen* very dark brown, the venter somewhat paler brown proximally ; densely clothed with very dark hairs. Spermathecae two, very dark and highly chitinised, pyriform but somewhat dissimilar in both the females examined (see fig. 2), the total length and greatest breadth of the one, 63μ by 50μ, and of the other, 77μ by 52μ in one specimen.

GOLD COAST : Accra, 1920 and 1921, 2 ♀♀, taken in the laboratory.

This insect resembles closely both *L. stylifer* and the preceding species, *L. nigeriae*, but differs from both in a number of characters. For example, the third segment of the palp is furnished with a sensory pit, but it is only a small one ; the first tarsal segments of the hind legs are relatively longer, nearly three times as long as the second ; and the spermathecae are two and pyriform.

LASIOHELEA LITORAUREA, sp.n.

Length of body (two females), 0·8 mm.; length of wing, 0·7 mm.; greatest breadth of wing, 0·27 mm. A small brown midge, not very dark coloured, with paler brown legs: without scales.

Head darkish brown. Eyes hairy; narrowing above, the margins contiguous, but the facets separated rather widely, by about 18μ. Clypeus, proboscis, and palpi brown. Palpi with first segment small, second and fourth sub-equal, not quite twice as long as broad, fifth small, shorter than the fourth with which it is united rather broadly, third about as long as the fourth and fifth together, slightly inflated in the basal half, without a sensory pit, but with a shallow depression bearing sensory hairs on its inner aspect. *Antennae* (fig. 3, A) brown; torus darker than the flagellum segments. Third

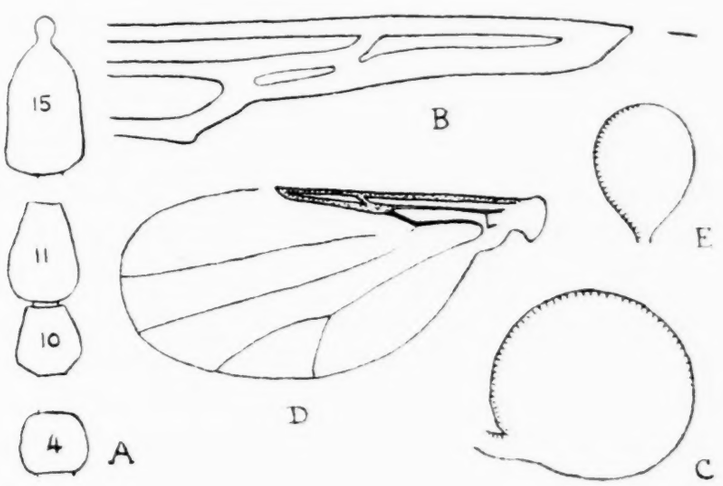

FIG. 3. *Lasiobelea litoraurea*, sp.n., ♀: A. segments 4, 10, 11, and 15, of antenna; B. radial cells; C. Spermatheca. *Lasiobelea inconspicuosa*, sp.n. ♀; D. diagram of wing venation; E. spermatheca. [D. × c. 80, B. × c. 265, others × c. 400.]

segment with a short stalk. Segments 4 to 10 sub-equal, sub-spherical, the fourth being slightly broader than long, the tenth slightly longer than broad; bearing stout spines which are about as long as the segments and are not very sharply pointed at their ends, and whorls of about twelve hairs. Segments 11 to 15 more elongated, the change of shape between the tenth and eleventh being definite; segments 11 to 14 sub-equal, length about once-and-a-half the breadth, the fifteenth slightly longer and broader and ending in a small knob. The terminal segments (11 to 15) not definitely

sculptured, but chitinised irregularly and so presenting a somewhat patterned appearance. The combined length of segments 11 to 15 about equal to that of segments 3 to 10, *e.g.*, in one specimen, 41 to 43 units. *Thorax* brown, the dorsum much darker than the pleura; sparsely clothed with hairs. Scutellum dark brown; bearing two lateral and five centro-marginal bristles, and about six small hairs. Post-scutellum dark brown. *Wings* (Pl. XXII, fig. 4) unadorned; well-clothed all over with minute microtrichia and longish hairs. Macrotrichia of the usual type, extending over practically the whole wing with the exception of the radial areas. There are, however, indistinct traces (especially along the fifth vein) of bare areas along the veins. At the base of the wing, between the fourth and fifth veins, are three or four rows of macrotrichia; at the level of the bifurcation of the fifth vein, about five rows between the fourth and fifth veins, about five rows between the rami of the fourth vein, and three rows between the third and fourth veins. It is difficult to determine exactly the number of rows because they are irregular, and because the rami of the fourth vein are very indistinct, indeed almost obsolete. The macrotrichia on the costa and anterior veins are longer and stronger than those elsewhere on the wing, but are not scale-like. The tip of the wing is rounded and very broad. Fringe well developed, composed of three rows of straight, simple hairs; the border of the wing thickened excepting for a short distance just beyond the end of the costa. Costa reaching beyond the middle of the wing, almost two-thirds of its length (22 : 36). First radial cell indistinct but not entirely obliterated, second long and narrow (fig. 3, B). Bifurcation of the fifth vein a little proximal to the end of the costa. Halteres pale brown. *Legs* brown, not so dark as the rest of the body, almost uniformly coloured; well clothed with hairs, but without scales. Femora not swollen, unarmed. Fore tibiae with a small patch of large, spine-like hairs near the apex; hind tibiae with the usual two transverse rows of bristles distally. First tarsal segment more than twice the length of the second on all the legs, on the hind legs nearly three times as long (37 : 13); distal segments short, sub-cylindrical. Claws rather slender, about half the length of the last tarsal segment. Empodium long and hairy. *Abdomen* brown, sides paler; clothed with shortish brown hairs. Spermatheca (fig. 3, C) single, sub-spherical, diameter about 50μ; duct arising obliquely and chitinised for a short distance.

GOLD COAST : Accra, 27.III.1920, 2 ♀♀, taken in the laboratory ; Christiansborg, near Accra, 14.II.1916, 1 ♀ (Dr. J. W. S. Macfie), ' taken in the Castle.'

LASIOHELEA INCONSPICUOSA, sp.n.

Length of body (one female), 0·9 mm. ; length of wing, 0·6 mm. ; greatest breadth of wing, 0·27 mm. A small brown midge very similar to the preceding species (*L. litoraurea*), but differing from it in the following respects :—

Head. Eyes bare ; facets separated only narrowly. *Antennae.* The flagellum segments form an almost continuous series, the change of shape between the tenth and eleventh segments being slight ; the basal segments slightly broader than long, the distal ones slightly longer than broad, the measurements of length and greatest breadth of the fourth, tenth, eleventh, and fourteenth segments being 5 by 7, 6 by 6, 7 by 6, and 7 by 6 units respectively. The last segment is slightly longer and broader than the others, about 12 by 7 units, and ends in a blunt knob. The combined length of segments 11 to 15 slightly greater than that of segments 4 to 10, but slightly less than that of segments 3 to 10, namely, 41 units as compared with 37 and 44 units. *Thorax.* Scutellum bearing two lateral and seven centro-marginal bristles, and about twelve small hairs. *Wings* (fig. 3, D). Shape of wing and distribution of macrotrichia as in *L. litoraurea*, but the microtrichia rather larger, and the first radial cell obliterated. *Legs* as in *L. litoraurea*. *Abdomen.* Spermatheca (Pl. XXII, fig. 5 and text-fig. 3, E) single, pyriform, length about 38μ, greatest breadth about 30μ ; the duct chitinised for a very short distance, about 3μ.

GOLD COAST : Accra, 1920, 1 ♀, taken on a window in the laboratory in the evening.

LASIOHELEA LEFANUI, (Carter)

NIGERIA : Minna, 14.VIII.1910, 2 ♀♀ (Dr. J. W. S. Macfie) ; Ilorin, old Residency, 29.VIII.1920, numerous ♀♀ (Dr. J. R. C. Stephens), ' biting at 1 p.m.' ; Mokwa, 16.XI.1921, several ♀♀ (Dr. J. R. C. Stephens), ' biting at noon.'

GOLD COAST : Accra, 1920, 1 ♀ ; Northern Territories, Bole, 26.VII.1918, Dogankade, 14.V.1918, Kulmasa, 22.VII.1918, Malowe, 29.VII.1918, Tanina, 21.VII.1918, and Ulu, 7.VII.1918, numerous ♀♀ (Dr. A. Ingram) ' taken in the act of biting.'

EXPLANATION OF PLATE XXII

Fig. 1. Wing of female of *Lasiohelea nigeriae*, sp.n.
Fig. 2. ,, ,, *L. brevitarsata*, sp.n.
Fig. 3. ,, ,, *L. caliginosa*, sp.n.
Fig. 4. ,, ,, *L. litoraurea*, sp.n.
Fig. 5. ,, ,, *L. inconspicuosa*, sp.n.

Figs. 1 to 3 from unstained, figs. 4 and 5 from lightly stained preparations.


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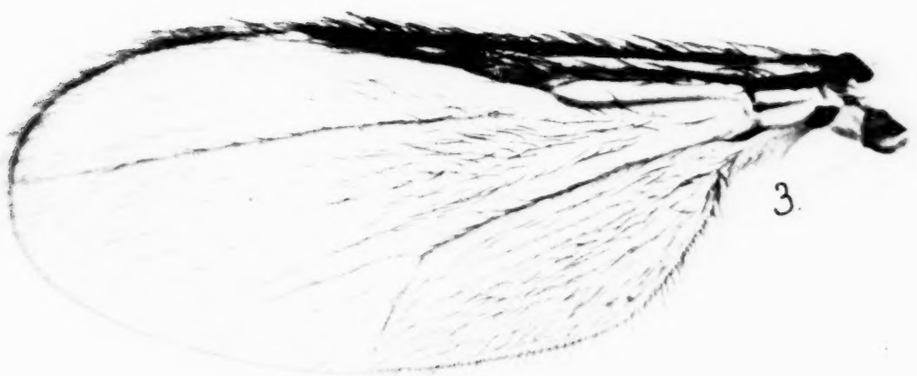
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A REVIEW OF THE OESOPHAGOSTOMES IN THE COLLECTION OF THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

BY

H. THORNTON

(*From the Parasitological Department of the Liverpool School of Tropical Medicine*)

(*Received for publication September* 15, 1924)

The material available for examination was as follows :—

1. *Oesophagostomum columbianum* from ileum of a Merino sheep.
2. *Oesophagostomum columbianum* from fourth stomach and small intestine of a sheep, Nairobi.
3. *Oesophagostomum columbianum* nodules from the small intestine of a sheep, Nairobi.
4. *Oesophagostomum columbianum* from duodenum of goat.
5. *Oesophagostomum columbianum* from a water buck, Ngoa, North-eastern Rhodesia.
6. *Oesophagostomum venulosum* from goat.
7. *Oesophagostomum venulosum* from lamb, Pwllheli, North Wales.
8. *Oesophagostomum radiatum* from caecum and large intestine of zebu.
9. *Oesophagostomum dentatum* from pig, Suffolk.
10. *Oesophagostomum mwanzae* from caecum and colon of wart hog (*Phacocaerus aethiopicus*), Rhodesia.
11. *Oesophagostomum eurycephalum* from caecum and colon of wart hog, Rhodesia.
12. *Oesophagostomum simpsoni* from caecum and colon of wart hog, Rhodesia.
13. *Oesophagostomum oldi* from caecum and colon of wart hog, Rhodesia.
14. *Oesophagostomum yorkei*, n.sp. from caecum and colon of wart hog, Rhodesia.
15. *Oesophagostomum ventri* n.sp. from stomach of Brazilian wild cat.
16. *Oesophagostomum brumpti* from chimpanzee (*Anthropopithecus* sp.)

Genus *Oesophagostomum*, Molin, 1861.

It is not proposed to use the subgenera *Hysteracrum*, *Oesophagostomum* and *Proteracrum* created by Railliet (1919) for the oesophagostomes of *Artiodactyla*. The discovery of several new species of the genus *Oesophagostomum* from the roan antelope (*Hippotragus equinus*) and the wart hog (*Phacocaerus aethiopicus*) has led one to realise the limitations of Railliet's subgenera. Neither Daubney's new species *O. mwanzae*, though it agrees most nearly with the subgenus *Proteracrum*, nor *O. eurycephalum* and *O. oldi* of Goodey (1924), can be included in any of these. It seems, then, that in regard to the genus *Oesophagostomum* one is fast approaching the stage where a subgenus will hold but a single species, obviously an untenable position. Goodey says of this :

> 'We have in the genus *Oesophagostomum* a neat and compact array of species which resemble the type species of the genus (*O. dentatum*) on broad lines, as for example in possessing a mouth collar with circumoral papillae, mouth with, or in one case, without external leaf crown, a cephalic vesicle generally inflated. The spicules and gubernaculum similar in structure and appearance, genital cone built on same general plan throughout all the species and ovijector apparatus also similar in all the species.'

On these grounds it is suggested that the genus be maintained intact. Further classification, if the number of species rendered the genus unwieldy, should include, in addition to the oesophagostomes of the *Artiodactyla*, those of Primates, Man, Rodentia, Carnivora and all other oesophagostomes without regard to the animal host. Grouping according to the animal host, as Railliet has done, is unsatisfactory, for there is a close relationship between *O. ventri* from the Brazilian wild cat and *O. stephanostomun* from man, and between *O. xeri* of the ground squirrel and *O. brumpti* of man.

Oesophagostomun columbianum (Curtice, 1890) Stossich, 1899.

Ransom (1911) says that this worm is found in the large intestine ; but it will be noticed that of the five lots of material collected, two from Nairobi, from Natal, from Queensland and North-eastern Rhodesia, all the worms were collected from the abomasum (fourth stomach) or small intestine. It appears to be the commonest oesophagostome of ruminants in Africa and America and is responsible for the so-called ' pimply gut ' of sheep. Theiler (1921) says ' very few sheep

pass our post-mortem table in which they are absent.' He further distinguishes two fatal sequelae to a severe infection, (1) a septic infection of the serous cavities due to the rupture of one or more intestinal nodules and, (2) an invagination of the ileum. To my knowledge severe infestations due to oesophagostomes, or such sequelae, are unheard of in England.

Descriptions of this nematode are given by Ransom, and more recently, by Goodey (1924). Curtice (1890) says of the two leaf crowns, 'There are twenty-four elements in each.' Giles agrees with him, but figures the inner row as typical bidentate elements. In the specimens I have examined there seems little doubt that the internal crown is composed of 48, twice as many elements as the external. Giles probably mistook two adjoining teeth for a larger bidentate one. *O. columbianum* has not previously been recorded from the water buck.

Oesophagostomum venulosum (Rudolphi, 1809) Railliet, 1896.

This is the commonest oesophagostome of the domesticated animals in England. Morgan says in his survey of the Aberystwyth area of Wales, '*O. venulosum* has been frequently found in the large intestine of sheep, but only in small numbers.' Ransom gives the number of elements in the external and internal leaf crowns as not more than sixteen processes in each worm. There is no doubt that, as in *O. columbianum*, the internal leaf crown is composed of twice as many elements as the external.

Oesophagostomum radiatum (Rudolphi, 1803) Railliet, 1898.

Some fifteen specimens were taken from the caecum and colon of the zebu. It has not been previously recorded from this host. Other recorded hosts are *Bos taurus* and the water buffalo. In the male bursa the common stem of the lateral rays projects dorsally, forming a protuberance at the root of the postero-lateral ray, an arrangement which is found in the bursae of all the oesophagostomes of the wart hog.

Oesophagostomum dentatum, Rud, 1803.

Fairly common in the caecum and colon of pigs in England, and when present they are usually very numerous.

Oesophagostomum eurycephalum, Goodey, 1924.

Recorded by Goodey from the roan antelope and found by me in material collected from the African wart hog (*Phacocaerus aethopicus*). It has not been previously recorded from this latter host.

My description and drawings, completed before the publication of Goodey's paper, agree closely with his. As regards the external leaf crown he says it is composed of eight stout elements whose tips curve outwardly, whilst in many of my specimens the tips projected vertically out of the mouth opening. Goodey states that the prebursal papillae are absent or so small as to be undiscernible, but I found these, though small, lying at the level of the anterior end of the gubernaculum. The caudal papillae are prominent, situated about 24μ from the tip of the tail. My measurements, agreeing in the main with those of Goodey, differ in some respects. Male length 15 mm. (Goodey 9 to 10 mm.) by 0·6 mm. in breadth. Length of oesophagus 705μ, posterior breadth 196μ. Breadth of buccal capsule 112μ, length 41μ. Distance of ventral slit from anterior extremity 512μ. Distance of cervical papillae from anterior extremity 426μ. Spicules are 1·49 mm. in length, cross-striated and 'winged' at their distal extremities. Female 16 to 17 mm. in length (Goodey 10 to 12 mm.) by 0·5 mm. in breadth. Oesophagus 750 to 900μ in length, posterior swelling 200 to 260μ in breadth. Buccal capsule 150μ in breadth by 50μ high. Vulva 380 to 420μ from tip of tail. Vagina 160 to 180μ in length, almost transverse. Ovijector stout, 320μ in length. Anus to tip of tail 130μ. Eggs 90μ by 56μ.

In the male bursa the outer terminal division of the dorsal ray is as thick as, but only one-quarter the length of, the inner division.

Oesophagostomum mwanzae, Daubney, 1924.

From caecum and colon of wart hog, Rhodesia. It is the commonest oesophagostome of the wart hog. Lateral cephalic papillae conical and blunt; submedian elongated, with a distal rod-like portion usually bent inwards. Buccal capsule ellipsoidal in cross-section. Seen from lateral aspect the walls diverge

anteriorly ; from the dorso-ventral aspect they appear to converge anteriorly. The external leaf crown is composed of six elements, each one long and pointed, arising from base of buccal capsule. There is no internal leaf crown. The cervical groove does not extend as far as the lateral lines. The intestine at its commencement is wider than the oesophagus, pigmented, though not so deeply as in *O. dentatum* and runs a straight course to the anus. Bursa has a well-defined dorsal lobe well separated from the two lateral lobes and is longer than them. The externo-dorsal ray is bent abruptly downwards at its middle. The medio-lateral and postero-lateral rays are joined and also the ventro-ventral and latero-ventral rays. The dorsal ray was unusually stout in the specimens I examined. The outer division of each main branch is as thick as the inner, but about half its length ; gubernaculum 100 to 120μ (Goodey 160μ) in length and trowel shaped. Daubney (1924) figures the handle portion as flexed dorsally, but in my specimens this part was practically straight. Seen laterally under high powers, the gubernaculum is produced at its distal extremity into a hook-like process.

The female possesses small caudal papillae, situated laterally about 30μ from the tip of the tail. My measurements differ slightly from those of Goodey. Length of male 11 to 12·2 mm. (Goodey 13 to 16 mm.), 430 to 470μ in breadth, ventral slit 280 to 295μ from anterior extremity (Goodey 0·2 mm., Daubney 0·22 mm.). Buccal capsule dorso-ventral diameter 100μ (Daubney 126μ).

Female 14·7 to 18 mm. in length (Goodey 16 to 20 mm.). Anus to tip of tail 125 to 176μ, vulva to tip of tail 300 to 360μ. Eggs segmented when laid, 64 to 71·4μ long by 34 to 37·4μ in breadth.

Daubney, in discussing the position of *O. mwanzae* in the genus, suggests that it shows near affinities to *O. apiostomum* and *O. brumpti*. I cannot agree with this, for I regard the shape of the buccal capsule in *O. brumpti* and *O. apiostomum* (that of a rather thin-walled truncated cone) as one of the chief characters and in *O. mwanzae* the walls, seen laterally, are ellipsoidal and diverge anteriorly. The new sub-genus, *Conoweberia*, created by Ihle (1922) to include *O. apiostomum* and *O. brumpti*, indicates the importance attached by him to the shape of the buccal capsule.

Goodey considers *O. mwanzae* most nearly akin to *O. simpsoni* in the elliptical shape of the head, leaf crowns, etc., but not in the

shape of the female tail and the oesophagus. This opinion is justified, for although he was in possession of only female specimens of *O. simpsoni*, my description of the male bursa of the latter shows that it bears a marked resemblance to that of *O. mwanzae*. In *O. simpsoni* and *O. mwanzae* the dorsal lobe is longer than the lateral lobes, the externo-lateral ray is closely applied to the medio-lateral at its origin and the outer border of the main stem possesses a rounded knob at the point of origin of the postero-lateral ray. This knob exists in all the bursae of the oesophagostomes occurring in the wart hog. Of the other oesophagostomes only *O. radiatum* possesses it.

Oesophagostomum simpsoni, Goodey, 1924.

The material consisted of some four males and twelve females, collected from the caecum and colon of the wart hog, Rhodesia—Goodey possessed no male specimens. A rather stout species with a breadth-length ratio of 1 : 21. The body tapers gradually from the anterior two-fifths towards each extremity, the anterior extremity being truncated. The mouth collar is very flat, almost disc like, and elliptical in cross-section. The dorso-ventral diameter of the buccal capsule is the greater, being 105 to 130μ, while the lateral diameter is 74 to 80μ. The external leaf crown is composed of eight stout elements arising from base of buccal capsule and projecting beyond the mouth collar, with slightly divergent points. There is no internal leaf crown.

The lateral cephalic papillae are very blunt and scarcely elevate the cuticle. The submedian are elongated, inclined inwards, and have a small knob-like distal portion. The cephalic vesicle is well-developed, giving the anterior extremity, under low power magnification, an appearance not unlike the head of a match.

The cervical groove is well-defined ventrally and extends as far as the lateral lines. The cuticular inflation is continued for a short distance beyond the groove, becoming lost opposite the commencement of the intestine. There are no lateral membranes, hence the stretched-out position of the worms when fixed. The cervical papillae, similar to those of *O. dentatum*, lie opposite the posterior swelling of the oesophagus.

The oesophagus is very short and broad, scarcely enlarging

posteriorly and flattened dorso-ventrally corresponding to the shape of the mouth capsule. Its breadth-length ratio is 1 : 2·2. In *O. dentatum* it is 1 : 3·5. At the entrance to the oesophagus there is no oesophageal funnel, but at this level the oesophagus presents a short sub-globular swelling, constricts slightly at the level of the nerve ring, finally enlarging slightly and projecting for a short distance into the intestine. The intestine at its commencement is only slightly larger than the oesophagus.

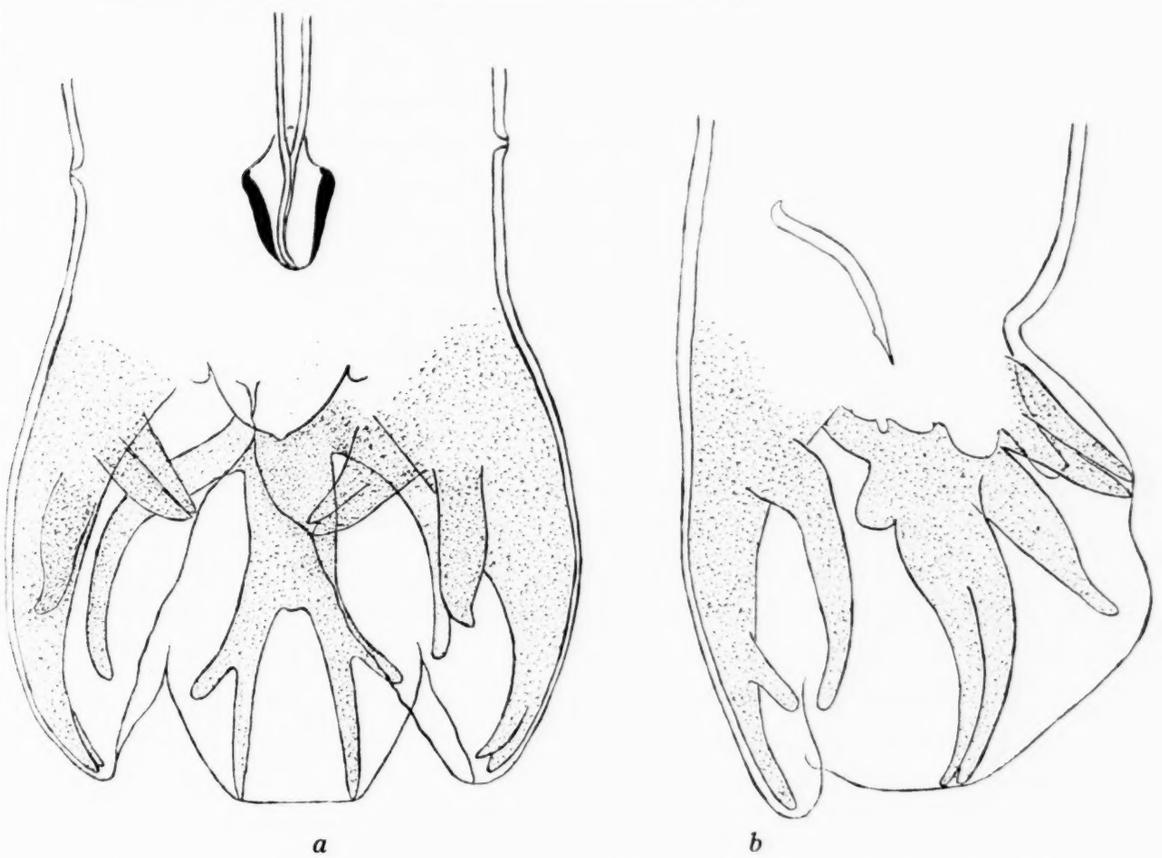

FIG. 1. *Oesophagostomum simpsoni*, Goodey. *a*.—Male bursa, ventral view ; *b*.—Same, lateral view.

The male worm has an average length of 15 mm. by 740μ broad. The oesophagus is 426μ long by 190 to 200μ broad. The ventral groove is 245μ from the anterior extremity and the cervical papillae 95μ behind this. The bursa has its dorsal lobe well separated on either side from the lateral lobes by a deep fissure, but the dorsal lobe itself is not deeply indented, though slightly longer than the lateral lobes. Prebursal papillae are present and lie opposite the anterior end of the gubernaculum. The bursal formula agrees

with that of the oesophagostomes. The ventral rays are normal and their tips reach the edge of the bursa. The externo-lateral is closely applied to the medio-lateral at its origin, then becomes well separated from it. Broader than either of the lateral rays at its origin, it becomes narrowed rather suddenly towards its extremity. The outer border of the main stem of the lateral rays, as in *O. radiatum*, *O. eurycephalum* and *O. mwanzae*, possesses a rounded knob at the point of origin of the postero-lateral ray. The lateral rays reach the edge of the bursa and their tips are strongly curved towards the dorsal rays.

The angle caused by the division of the main dorsal ray is acute ; the outer terminal division of the dorsal ray is as thick as, but less than half the length of the inner terminal division and is inclined outwards. The gubernaculum is trowel-shaped, similar to that of *O. dentatum*. It is 130 to 160μ long and not markedly bent in its centre. Seen laterally the distal extremity is barbed on its dorsal aspect. The spicules are equal, alate, and appear fused at their tips.

The female worm is 15 to 21 mm. long, 0·7 to 1·05 mm. broad. The oesophagus is 430 to 460μ long by 210 to 220μ broad. The ventral slit is about 230μ from the anterior extremity, the cervical papillae 100μ behind this. Behind the genital opening the tail is straight and constricts gradually to the anus, terminating behind this in a conical point, on either side of which lies a caudal papilla. The vulva is salient and the vagina is about 150μ long and transverse. There is a double muscular ovijector about 320μ long from which two uteri run forwards. The vulva is 500 to 700μ in front of the posterior extremity, the anus 140 to 170μ from the tip of the tail.

Oesophagostomum oldi, Goodey, 1924.

In examining the wart hog material, I had found and described a worm agreeing closely with Goodey's new species *O. oldi*. In the main my description and measurements agree with his, but there are several differences I wish to record.

The cephalic vesicle is but slightly developed ; anterior to the cervical groove are a number of lesser circular grooves three to four in number which indent the cuticle. Goodey makes no mention of these. As the worms had been excellently preserved I cannot regard these grooves as due to shrinking of the cuticle.

Goodey notes that the external leaf crown does not arise from the base of the buccal capsule. In my specimens it takes its origin from the lower fifth of the wall.

The male measures 9·3 to 12·4 mm. in length (Goodey 11 to 13 mm.). Female 7 to 14 mm. (Goodey 15 to 18 mm.).

The tail of the female bears resemblance to the human foot, though in my specimens there is a sharp depression, not figured by Goodey, which separates the vulva and anus. Caudal papillae are prominent and slightly asymmetrical, 80 to 140μ from the tip.

The constant difference between my specimens and his lies in the length of the vagina. It is very long in both, but while Goodey gives its length as 0·8 to 1 mm., it measured in my specimens an average of 1·6 mm. and never less than 1·3 mm. The other characteristics, however, agree so closely with *O. oldi* that on these differences one is hardly justified in founding a separate species.

Oesophagostomum yorkei, n.sp.

Four females and two males were collected by Professor Yorke in Rhodesia, from caecum and colon of *Phacocaerus aethiopicus*.

Male about 10 mm. long by 0·4 mm. in breadth, females 14 to 15·3 mm. long by 0·65 mm. in breadth.

Mouth collar inflated, slightly elliptical in cross section. The cephalic papillae have their usual disposition, laterals blunt, submedian, long and prominent.

The cephalic vesicle is scarcely inflated. Cervical groove extends from ventral surface as far as lateral lines and is 240 to 260μ from the anterior extremity. Between the cervical groove and the head are three or four lesser grooves which indent the cuticle. The cervical papillae are like those of *O. dentatum* and are about 330μ from the anterior extremity. The mouth opening is slightly elliptical in cross section. There is an external leaf crown of eight large elements whose points project vertically beyond the mouth opening. These elements arise from the base of the inner wall of the mouth capsule. There is no internal leaf crown. The buccal capsule is about 40μ in height by 55μ in breadth. The walls in optical section appear parallel and ellipsoidal, but are constricted in their anterior fourth to form a neck. The body wall is connected by strands with the posterior aspect of the buccal capsule.

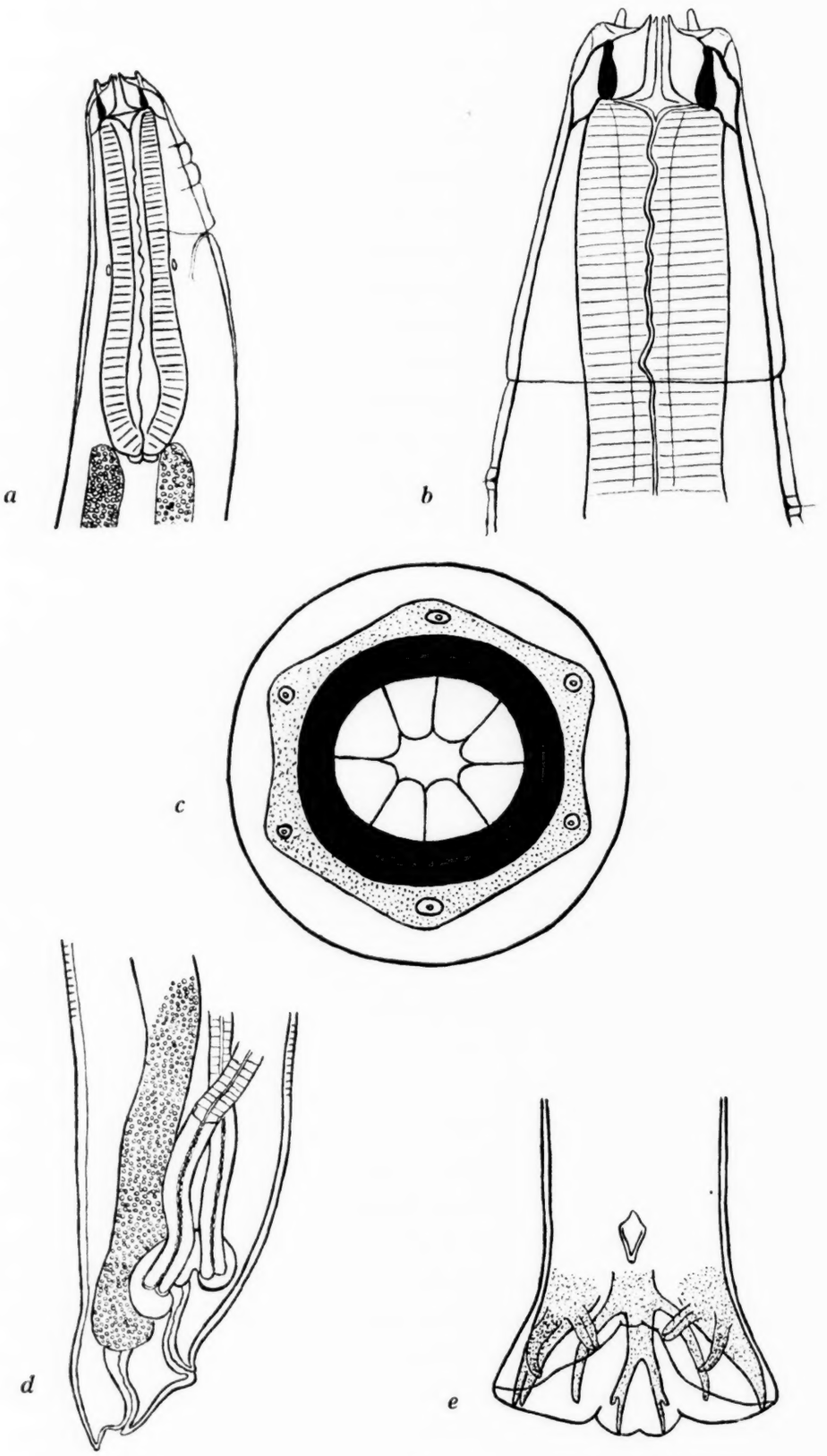

FIG. 2. *Oesophagostomum yorkei*, n sp. *a*.—Anterior end, lateral view; *b*.—Same, ventral view, more highly magnified; *c*.—End-on view of head; *d*.—Tail end of female; *e*.—Male bursa, ventra view, somewhat flattened.

The oesophagus is about 550μ in length, of equal thickness in its anterior three fourths; it then dilates, being here about 150μ in breadth. Oesophageal-intestinal valves small. There is an oesophageal funnel about 40μ deep lined by cuticle, but there are no teeth. The nerve ring is just behind the cervical groove.

The body is of equal width as far as the vulva, then tapers abruptly to a small, straight, conical tail. The vulva has salient lips and is about 260μ from the tip of the tail. The vagina is 190 to 210μ in length, has stout walls and is directed forwards to a double muscular ovijector about 240μ in breadth. From it two uteri run directly forwards. The rectum is about 190μ long and the anus 80μ from the tip of the tail.

Male. The bursa is deep and not expanded to a greater width from the rest of the body. The dorsal lobe is separated from the lateral lobes on either side by a fissure and is slightly indented in the middle line. The terminal divisions of the main dorsal ray are slender and the outer terminal division is directed straight backwards. As in *O. oldi* and *O. eurycephalum* there is a distinct rounded knob on the posterior border of the main lateral trunk at the origin of the postero-lateral ray; externo-dorsal rays given off close to base of dorsal ray and are long and slender. Spicules are alate with their tips bent dorsally; they are short, 1·3 mm., corresponding to shortness of vagina in female. The gubernaculum is shovel-shaped, 130μ long.

This worm appears to occupy an intermediate position as regards its relationship to the other oesophagostomes of the wart hog; it resembles *O. oldi* in the shape of the oesophagus and the presence of a well-marked oesophageal funnel without teeth. Also in the shape of the buccal capsule and the shape and structure of the male bursa. It bears, however, an even closer resemblance to *O. eurycephalum* in the number of elements in the leaf crown and, in regard to the tail, the abrupt narrowing behind the vulva, the short almost transverse vagina and the vulva with salient lips.

This parasite has been named in honour of Professor Warrington Yorke of the Liverpool School of Tropical Medicine.

Oesophagostomum ventri, n.sp.

These worms, consisting of two males and fifteen females, were obtained by Dr. Thomas from the stomach of a Brazilian wild cat, together with numerous specimens of *Toxascaris marginata*. Taking into account the fact that oesophagostomes are found usually in the caecum and large colon and that available literature shows no record of the wild cat as a host of this genus, one was naturally led to the assumption that these worms were spurious parasites the worms being found in the intestine post-mortem—owing to the cat having eaten the intestine of some other animal a short time before death.

Against this contention is the fact that the worms were in an excellent state of preservation, while the presence, in the stomach also, of specimens of *Toxascaris marginata* would serve to strengthen the evidence that these oesophagostomes were true parasites of the cat.

This worm, then, is regarded as a new species from the cat. Further evidence may show this new oesophagostome to be normally parasitic in some other animal, possibly one of the smaller rodents, which then becomes the true host.

Description. The worms are whitish, body practically straight, of maximum diameter at the junction of the middle and anterior fifths and diminishing gradually towards each extremity. The anterior end is truncated, the mouth collar being broad and shallow and separated from the body by a well-defined groove. There are six head papillae. The laterals are broad and flat and scarcely protrude above the surface. The sub-median are conical, not very prominent and the rather delicate pulp projecting into them from below gives each papilla in optical section an appearance not unlike the head of an arrow.

The buccal capsule is shallow, its height to breadth ratio being 1:4. The walls in section appear ' comma ' shaped, thicker anteriorly and convex on their inner aspects. From the base of the wall a chitinous ridge runs downwards and outwards to reach the body wall.

The external leaf crown arises from the anterior border of the buccal capsule. It is composed of some 45 slender pointed elements

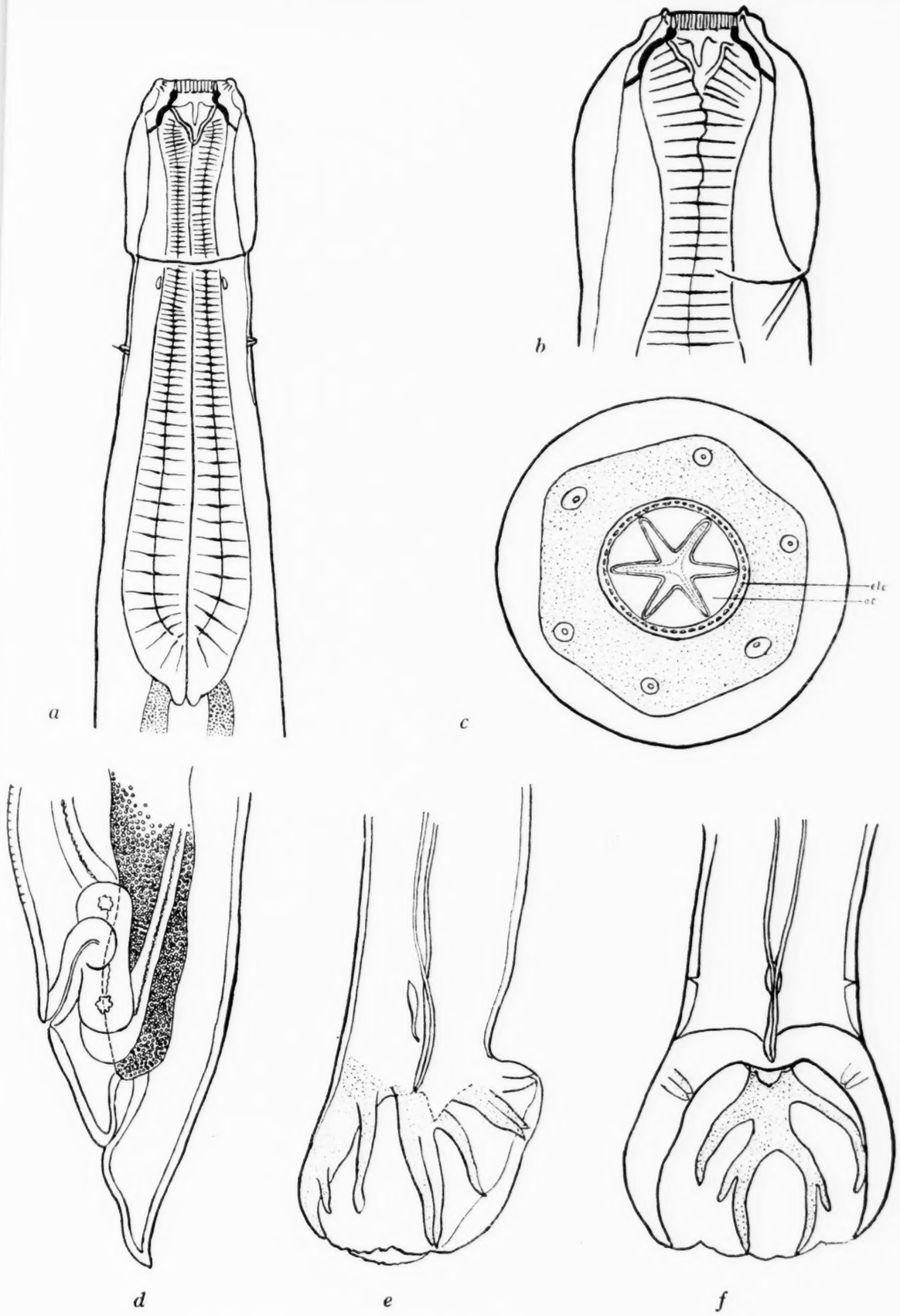

FIG. 3. *Oesophagostomum ventri*, n.sp. *a.*—Anterior end, seen ventrally; *b.*—Same, lateral view, more highly magnified; *c.*—End on view of head; *e.l.c.*—External leaf crown, *o.t.*—oesophageal tooth; *d.*—Tail end of female; *e.*—Male bursa, lateral view; *f.*—Same, ventral view.

projecting directly forwards, but not extending beyond the mouth opening. There is no internal leaf crown. The cephalic vesicle is well-defined, constricted inferiorly by a groove extending from the ventral surface as far as the lateral lines. The excretory duct opens at this level and the nerve ring is found just behind this.

There is a large oesophageal funnel whose chitinous lining is modified to form six large teeth, their slightly acute points being directed upwards and inwards. Two teeth correspond to each oesophageal sector. The tooth of one sector is widely separated from its fellow, but lies in apposition with the tooth of the adjoining sector.

The oesophagus is long, narrows directly behind the oesophageal funnel, then gradually dilates to its posterior fifth. The cervical papillae lie just anterior to the middle of the oesophagus.

Female. Length 20 to 23 mm. by 670 to 700μ broad. The cuticular striations are about 17μ distant at the centre. The oesophagus is 1·23 to 1·26 mm. in length, posterior breadth about 260μ. The buccal capsule is about 80μ broad by 20μ high. Distance of ventral slit from anterior extremity 330 to 360μ. Cervical papillae about 540μ from anterior extremity. Caudal papillae are present on either side of the tail about 50μ from the tip. The tail is straight and slender, tapering gradually behind the vulva. The tip of the tail is sometimes bent dorsally.

The vulva is salient about 560μ from the tip. Vagina almost transverse, 190 to 260μ in length, and runs to a double muscular ovijector 200 to 270μ in length. From its extremities run two uteri, the anterior running directly forwards, the posterior running backwards, but curving almost immediately to run forwards again. The anus is non-salient, 180 to 260μ from the tip of the tail.

Male. About 21 mm. in length, 690μ broad. Length of oesophagus 1·23 mm., posterior breadth 230 mm. Buccal capsule 78μ broad by 19μ high. Distance from anterior extremity to ventral slit 340μ, to cervical papillae 508μ. The bursal formula agrees with that of the genus *Oesophagostomum*. The left externo-dorsal ray becomes detached from the main dorsal branch at about its own width above the right. The inner and outer terminal division of the dorsal ray are both slender, the former being about three times as long with slightly incurved extremities. The externo-

lateral ray is well separated from the medio-lateral throughout its length and its tip reaches nearer to the edge of the bursa than does the externo-dorsal. The spicules are equal, tubular and non-alate, with fused tips. They are 1·32 to 1·47 mm. in length. The gubernaculum, unlike that of *O. dentatum*, is lozenge-shaped, about 135μ in length. Seen laterally it is not bent markedly at its middle, but is almost straight and tapers from above. Prebursal papillae are present at the level of the gubernaculum.

In considering the relationship between this worm and the other oesophagostomes one was struck with the remarkable similarity between it and *Oesophagostomum stephanostomum* var. *Thomasi*. These latter parasites were obtained by Dr. Thomas from the intestine of a Brazilian prisoner who died in Manáos. Both are indigenous to Brazil. Railliet and Henry consider Thomas's oesophagostome, *O. dentigerum*, Railliet and Henry, 1906, from the chimpanzee and *O. stephanostomum*, Stossich, 1904, from the gorilla, are simply varieties of the one species. The only constant difference between them lies in the disposition of the terminal divisions of the posterior ray in the male bursa. With Clayton Lane I would hesitate to attach even subspecific value to this character.

Comparison with these types leads one to state that *O. ventri* is a separate species differing from *O. stephanostomum* var. *Thomasi* in the following respects :—

Table I

	O. stephanostomum var. *Thomasi*	*O. ventri*, n.sp.
External leaf crown	38 elements	45 elements
Internal leaf crown	76 small elements	None
Sub-median head papillae ...	Rounded, but tip produced to sharp point	Conical
Buccal capsule	Ellipsoidal in optical section ...	'Comma'-shaped in optical section
Spicules	Tips free	Tips fused
Posterior bursal ray	Outer terminal division incurved	Outer terminal division parallel to inner
Oesophageal teeth	Six teeth equally spaced ...	Six teeth arranged in three pairs

In all other respects, *e.g.*, length of spicules, position of cervical papillae, shape of tail, position of vulva and length of vagina, *O. ventri* corresponds closely with *O. stephanostomum* var. *Thomasi*.

It would seem that this carnivoran oesophagostome bears a much closer relationship to the oesophagostomes of Man and Primates than it does to the oesophagostomes of Rodentia and the Artiodactyla.

I propose for this worm the specific name *O. ventri* to mark the unusual location in the host in which it was found.

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SAUROSITUS AGAMAE, N.G., N.SP. A FILARIOID PARASITE OF THE LIZARD *AGAMA COLONORUM*

BY

J. W. S. MACFIE

(*Received for publication* 19 *September*, 1924)

This worm is a common parasite of the lizard *Agama colonorum* in West Africa, the adults being found in the mesentery and the embryos in the blood. It may be noted here that the distribution of filariasis of lizards in West Africa is not uniform, for whereas two species are commonly found at Yaba, near Lagos, only one species, namely that referred to above, has hitherto been found at Sekondi (Dr. J. F. Corson), and none has yet been discovered at Accra, although the lizards there have been repeatedly examined at all seasons of the year.

SAUROSITUS AGAMAE, n.g., n.sp.

Body thread-like, colourless, tapering slightly towards the posterior extremity. Anterior extremity rounded, bearing two lateral and four sub-median papillae which, however, are very small. Cuticle smooth, not striated transversely, and without either cuticular bosses, annulations, or spiral thickenings. Mouth terminal, small, slightly projecting, without lips. Oesophagus short, divided into two unequal portions, the anterior short and narrow, the posterior three or four times as long, broader, not dilated at its junction with the intestine. Nerve ring well developed. Anus sub-terminal; tail short and blunt in both sexes.

Male. Shorter and more slender than the female; length (three specimens) 42 mm. to 55 mm., breadth at the middle of the body about 200μ. Posterior extremity coiled in a close, flat spiral. Cloacal opening about 15μ from the tip of the tail. Tail short and blunt; caudal alae absent. There are four pairs of pre-anal papillae,

but no para-anal or post-anal papillae. Spicules two, sub-equal, about 100μ to 130μ in length ; gubernaculum small.

Female. Length (three specimens) 80 mm. to 140 mm. ; breadth at the middle of the body about 300μ. Posterior extremity somewhat attenuated and terminating in two more or less distinct lobes, but without papillae. Rectum atrophied, anal orifice absent. Vulva situated just posterior to the oesophagus. Opisthodelphes. Ovoviviparous. Embryos in the blood.

Microfilaria. The embryos, which have been briefly described on a previous occasion (*Annals of Trop. Med. & Parasit.*, Vol. VIII, pp. 456-458, and Pl. XXV, figs. 1, 3, and 4, 1914), are enclosed in an ample sheath. The cuticle is striated. The nuclei are large, two or three abreast in the middle of the embryo, and almost completely filling the greater part of the body. The lengths of thirty specimens measured ranged from 106μ to 168μ, average 135μ. The breadth at the widest part is about 3μ. The body does not taper towards the anterior extremity and is bluntly rounded at its end. There is usually a small area, about 4μ long, at the anterior extremity which is free from nuclei. The nerve ring is situated about 28 per cent. of the length of the body from the anterior extremity : it is a narrow, usually oblique break. The excretory pore, G1 cell, and anal pore are situated respectively 41, 74, and 88 per cent. of the length from the anterior extremity. The body tapers posteriorly to a slender tail which terminates somewhat bluntly and is occupied by nuclei to its extremity.

In addition to the embryos there occur in the blood numerous small bodies which appear in stained specimens as I-shaped structures surrounded by a cuticular envelope. These bodies are also present in the uterus of the female, and are perhaps abortive eggs.

The embryos in the blood do not exhibit periodicity and were found to be present in approximately equal numbers in blood taken from the tail at 5 a.m., 10 a.m., 5 p.m., and 10 p.m.

The distribution of the embryos in the body was studied in a lizard which had been sent to Accra from Sekondi and had died one afternoon at about 4 p.m. soon after its arrival. The tissues of this lizard were preserved and cut into sections 0·014 mm. thick, and in these sections the filarial embryos in fifty microscope fields, each 100μ square, were counted with the results shown below :—

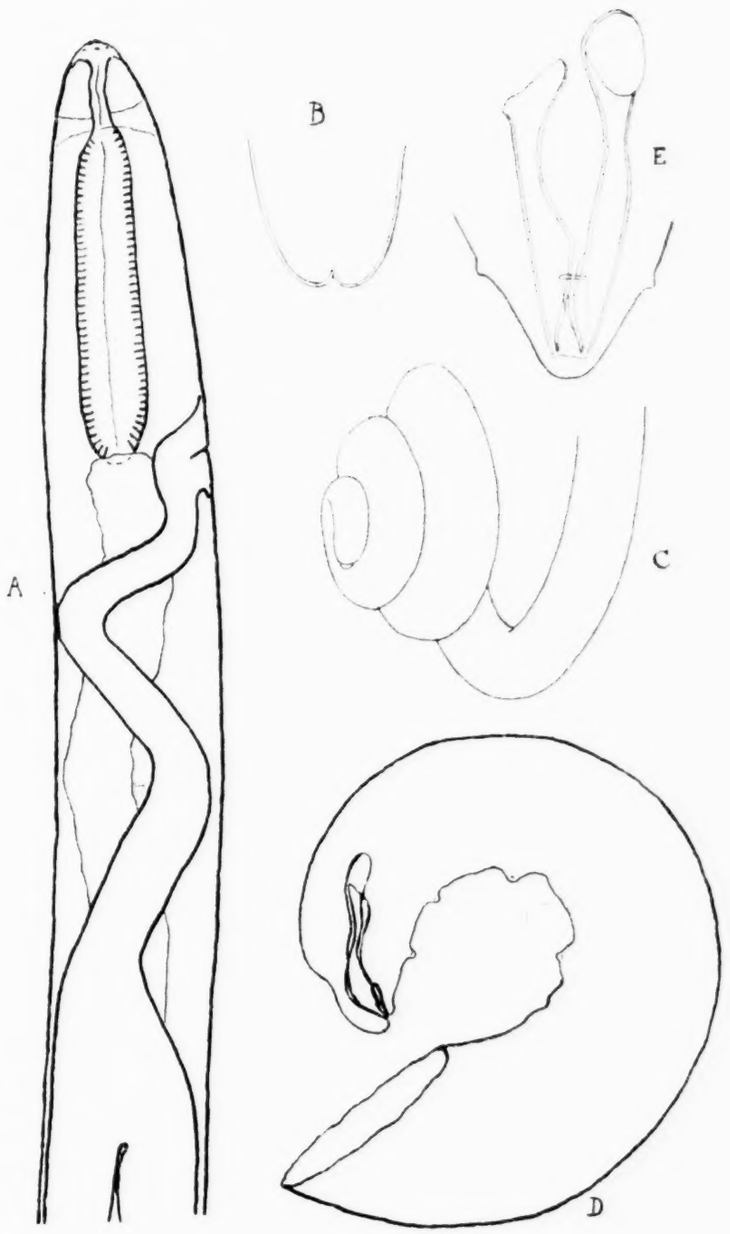

FIG. 1. *Saurositus agamae*, n.g., n.sp. *A.*—Anterior extremity of female, lateral view. × 45. *B.*—Posterior extremity of female, ventral view. × 150. *C.*—Posterior extremity of male. × 60. *D.*—Same, lateral view. × 150. *E.*—Posterior extremity of male, ventral view to show the spicules. × *c.* 300.

TABLE 1

Tissue	Filarial larvae in 50 fields
Lung	152
Liver	51
Spleen	28
Blood (sinus venosus)	5
Heart muscle	1
Muscle at base of tail	0
Testis	0

In the absence of accurate data, which are not available, as to the proportion of the various organs which is composed of blood, it is not possible to decide to what extent these figures indicate a concentration of the embryos in certain organs. With regard to the lung which is little more than a trellis for blood vessels, however, a study of the sections showed the embryos much kinked and twisted, and it was clear that such relatively large and long parasites could not flow freely through the vessels with the blood stream, and that, therefore, the lung must act as a filter, allowing the blood to pass more readily than the filarial embryos.

DIAGNOSIS. It is necessary to erect for this parasite a new genus the characters of which may be defined as follows :—Mouth without lips. Anterior extremity rounded, bearing six small papillae, two lateral and four sub-median. Cuticle smooth. Oesophagus divided into two portions.

Male. Posterior extremity coiled into a close spiral, tail short ; caudal alae absent, four pairs of preanal papillae ; spicules sub-equal, gubernaculum small.

Female. Vulva near the posterior extremity of the oesophagus ; anal orifice absent ; opisthodelphes. Microfilariae sheathed, in the blood.

HOST. Lizard, *Agama colonorum.*

LOCALITY. Nigeria and Gold Coast, West Africa.

MISCELLANEA

CONTORTOSPICULUM RHEAE

Whilst dissecting a *Rhea americana* brought to this country from South America twelve years ago, three large worms were found coiled up on the right side of the anterior abdominal air sac. The two larger worms, which were females, measured, respectively, 112·5 cms. and 101 cms., whilst the third (a male) was 20 cms. in length. No other worms were found in the air sacs, but two specimens (male and female) were embedded in the peritoneal tissue. On examination the worms were identified as *Contortospiculum rheae* (Owen, 1843), Synonym :—*Filaria horrida*, Diesing, 1851.

21 *June*, 1924.

J. Isgaer Roberts,
Bangor University.

XENOPSYLLA ASTIA, Rothsch

Correction of Record of its occurrence in West Africa.

The specimens recorded as *Xenopsylla astia*, Rothsch., from Accra, Gold Coast (Evans, 1922, *Ann. Trop. Med. & Parasit.*, Vol. XVI, p. 449) have been submitted to Mr. F. S. Cox, who identified them as the closely allied *Xenopsylla nubicus*, Rothsch.

A. M. Evans.

A CASE OF SLEEPING SICKNESS *(T. GAMBIENSE)* TREATED WITH 'BAYER 205'

H.L.S.

January, 1922. Trypanosomes found in blood in Nigeria.

May 28, 1922. Trypanosomes in blood.

Between May 30 and June 27, 1922, patient received 4 grammes of 'Bayer 205,' intravenously.

September 30, 1924. Remains well, *i.e.*, after 2 years and 3 months.

J. W. W. Stephens.

UNIVERSITY OF LIVERPOOL

December 28, 1924 Vol. XVIII. No. 4.

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POLYMORPHIC TRYPANOSOMES OF THE *T. BRUCEI* GROUP RECOVERED FROM THE MWANZA SLEEPING SICKNESS AREA

BY

H. LYNDHURST DUKE, O.B.E., M.D., Sc.D. (Cantab.),

BACTERIOLOGIST, UGANDA PROTECTORATE.

(*Received for publication 9 August*, 1924)

CONTENTS

INTRODUCTION

The trypanosome strains dealt with in this paper were recovered from the Sleeping Sickness area in the neighbourhood of Mwanza in Tanganyika Territory.

During a tour through the fly-belt in July and August, 1922, I isolated four different strains, all of which were inoculated into experimental monkeys. The strains included two from man,

one from wild fly, and one from wild game. The monkey carrying the wild game strain escaped; the other strains reached the Laboratory at Entebbe, and form the subject of the studies here set forth.

Several papers have been published dealing with the Mwanza outbreak of the human trypanosomiasis (Swynnerton, 1923 a and b; Duke, 1923a). The disease in man was generally acute and fatal, and, in places, attained epidemic proportions. The causative trypanosome, when inoculated into guinea-pigs, showed posterior-nuclear forms, and was accordingly diagnosed as of the nature of *T. rhodesiense*, rather than *T. gambiense*. The outbreak presented many points of interest. The obscurity surrounding the origin of the human parasite; its apparently sudden appearance, and its mode of propagation; the occurrence of a *T. rhodesiense* like organism in epidemic form, and so far north; the discovery that the insect vector was a new species, *G. swynnertoni*—all these features combined to produce a novel and fascinating problem.

The first part of the present paper deals with the three strains during their sojourn at the Entebbe Laboratory. They have been studied in relation to both the mammalian and the insect host. Incidentally, in the course of these studies, further information has been obtained about the behaviour of trypanosome strains when subjected to direct transmission over a prolonged period.

Part II represents an attempt to assemble and review the data hitherto obtained by the experimental study of directly-transmitted strains, and to set forth the conclusions resulting from these studies.

PART I

A.—ACCOUNT OF THE MAINTENANCE OF THE STRAINS AND THEIR ANIMAL REACTIONS

(1) HUMAN STRAIN, 483.

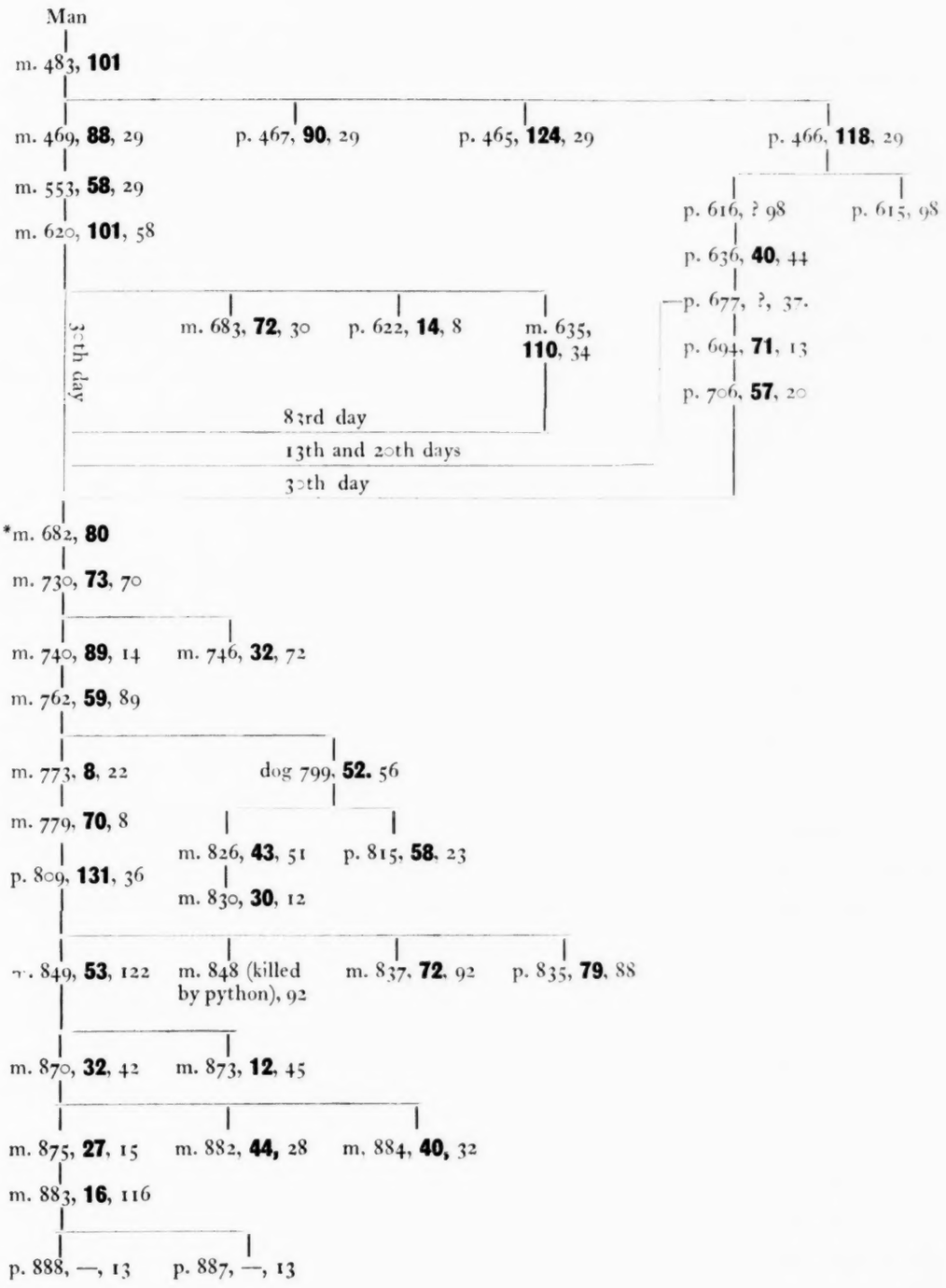
Inoculated on 1.8.22 from young adult native S. of Nyamagana village in the fly area.

The upkeep of this strain is shown in Table I.

The figures in black type give the duration of the disease in the animal; the other figure gives the day of the disease in the infecting animal when the sub-inoculation was made.

TABLE I.

Human Strain 483.



* Monkey 682 received in all 5 inoculations, as shown in the Table.

NOTE. In this and other similar Tables m. signifies monkey, and p. guinea-pig.

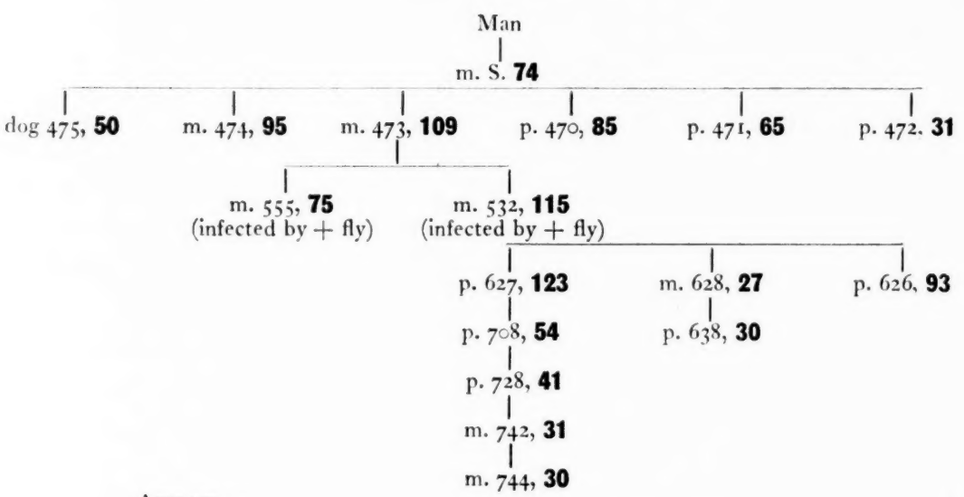
(2) HUMAN STRAIN S.

Monkey S. was inoculated on 2.8.22 from an adult male native M., of Gamboshi village in the fly-belt.

Table II shows the upkeep of this strain until July, 1923, when circumstances necessitated its abandonment.

TABLE II.

Human Strain S.



AVERAGE :—

8 completed monkeys	69.5 days.
8 completed guinea-pigs	65.2 days.
1 dog	50 days.

A young chimpanzee was fed upon by infective flies in Experiments 527 and 530 (of Tables IV and V), on two consecutive days. Trypanosomes were seen in its blood six days later, and it died sixty days after infection. This chimpanzee seemed quite well up to the day when its companion died (*cf.* below) ; from then on it began to mope and refuse its food. It is difficult to decide to what extent grief influenced the course of the infection.

Both the natives from whom these two human strains were derived denied knowledge that they were sick ; they were detected at the inspection musters held at villages in the fly-belt. They both had enlarged cervical and axillary glands, and looked as though they had recently lost flesh. The disease was, to all appearances, not very advanced. The Senior Commissioner, Mwanza, informs me that native S. died on 12.2.23, and native M. during December, 1922.

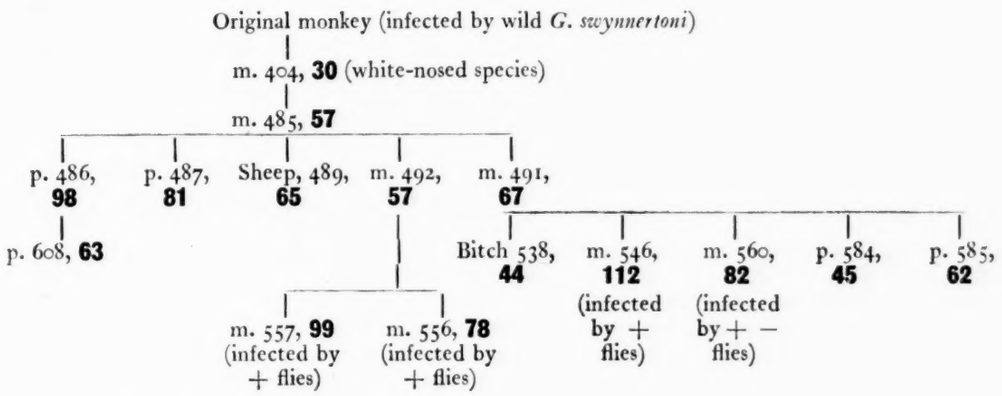
(3) Wild Fly Strain, E.

Recovered by feeding wild *G. swynnertoni* on a clean experimental monkey. Trypanosomes appeared first in the monkey's blood on 26.7.22.

Table III shows the maintenance of this strain, which was relinquished in January, 1923.

Table III.

Wild Fly Strain E.



Average :—

7 monkeys	78.8 days.
5 guinea-pigs	69.8 days.
1 bitch	44 days.
1 sheep (native)	65 days.

An old chimpanzee, fed upon by flies (Experiment 531, Tables VIII and IX), on a single occasion, became infected and died forty-eight days later. Four infective flies fed on this animal, and trypanosomes were already numerous in the peripheral blood on the ninth day after infection.

It is plain from Tables I, II and III that the three strains, when first isolated, were essentially identical in their behaviour in laboratory animals. Nor is any difference discernible in the morphology of the strains.

(4) Posterior-nuclear Forms :—

All these strains have been examined at intervals for posterior-nuclear forms, two hundred trypanosomes being inspected in each of a series of stained thin blood-films from infected guinea-pigs. In all the strains these forms were found, though latterly not at

every examination. In some guinea-pigs they were less common than in others. In guinea-pig 809 of Table I they were generally difficult to find. In the case of strain 483, posterior-nuclear forms are apparently less common now than formerly ; for example, they were always common in guinea-pig 835 of Table I, but rarer and often of less pronounced type in guinea-pigs 887 and 888.

Thus the Mwanza human strain 483 can still produce posterior-nuclear forms, though it has lost the power of cyclical development in tsetse ; it is, however, not quite clear whether the two processes are entirely independent. Now it is interesting to note that both the Direct Transmission Strain and the Antelope Strain ceased to produce posterior-nuclear forms in guinea-pigs in the later stages of the upkeep of these strains at the laboratory. It has been suggested elsewhere (Duke, 1921) that these two strains are descendants of trypanosomes which, at the time of the great epidemic in Uganda, were capable of infecting man, and that they have assumed certain of the characteristics of *T. brucei* as the result of long-continued and intimate association with *Tragelaphus spekei*. On the other hand, the Mwanza human trypanosomes are indistinguishable by laboratory tests from *T. brucei*. It is tempting to see, in the observations just recorded, confirmation of these views on the origin of the Uganda and Tanganyika strains, respectively. The power exhibited by the two former of producing posterior-nuclear forms, being a comparatively recent acquisition, is soon lost ; whereas, in the case of the Mwanza strain, the characters of typical *T. brucei* are more deeply rooted, and so persist longer under radical changes of environment.

These studies also show that increase of the virulence of a strain of trypanosomes is not necessarily associated with the formation by the strain of posterior-nuclear forms in suitable animals.

B.—ACCOUNT OF TRANSMISSION EXPERIMENTS CARRIED OUT WITH LABORATORY-BRED *G. PALPALIS*

(1) HUMAN STRAIN 483.

Table IV sets forth transmission experiments carried out with this strain shortly after its arrival at the laboratory, and Table V gives the results obtained by dissecting the positive flies obtained in these experiments.

TABLE IV.

Experiment No.	Infecting monkey	Date	Flies dissected			Day on which dissection began	Alive 25th day	Result of feeding on clean monkey	Date at which box became infective	Flies containing flagellates	Duration of experiment
			Males	Females	Total						
503	468	11–13.9.22	22	23	45	12	42	+	26	1	38
527	469	21–23.9.22	26	34	60	8	54	+	18	9	36
530	468	23–25.9.22	35	32	67	7	59	+		7	34
539	469	26–28.9.22	37	31	68	11	66	+	20	3	32
*583	469	11–12.11.22	23	35	58	16	54	—	—	2	66
*586	469	12–13.11.22	26	31	57	16	53	—	—	6	66
*588	469	13–14.11.22	35	31	66	23	63	—	—	3	64

The brackets indicate that the two boxes were fed upon the same clean monkey on alternate days.

NOTE.—The * indicates that the flies in these three boxes were fed throughout the experiment upon a baboon, an animal not susceptible to this strain of trypanosomes.

TABLE V.

Experiment No.	No. of fly containing flagellates	Day of experiment on which fly was dissected	Intensity of infection		Sex of fly
			Gut	Glands	
503	1	38	+++	+++	Male
527	2	27	+++	+	Female
—	3	32	+++	+++	Male
—	4	32	+++	+++	—
—	5	33	+++	++	—
—	6	36	+++	+++	—
—	7	36	+++	+++	Female
—	8	36	+++	+++	—
—	9	36	+++	+++	—
—	10	36	+++	—	—
530	11	7	++	—	—
—	12	7	++	—	—
—	13	34	+++	+++	Male
—	14	34	+++	+++	—
—	15	34	+++	+++	—
—	16	34	+++	+++	Female
—	17	34	+++	+++	—
539	18	32	+++	+++	Male
—	19	32	+++	+++	Female
—	20	32	+++	+++	—
583	21	15	+++	—	Male
—	22	40	+++	—	—
586	23	41	+	—	—
—	24	48	+	—	—
—	25	48	+	—	—
—	26	66	+++	+++	—
—	27	66	+++	+++	Female
—	28	66	++	—	—
588	29	39	+++	++	Male
—	30	44	+++	+++	—
—	31	62	+++	+++	—

(2) HUMAN STRAIN S.

Table VI gives the transmission experiments with this strain, and Table VII the results of dissecting the positive flies obtained.

TABLE VI.

Experiment No.	Infecting monkey	Date	Flies dissected			Day on which dissection began	Alive 25th day	Result of feeding on clean monkey	Date at which box became infective	Flies containing flagellates	Duration of experiment
			Males	Females	Total						
521	473	19–21.9.23	31	28	59	10	46	+	25	2	32
528	473	22–24.9.23	30	31	61	10	44	+	19	7	29

TABLE VII.

Experiment No.	No. of flies containing flagellates	Day of experiment on which fly was dissected	Intensity of infection		Sex of fly
			Gut	Glands	
527	1	32	+++	+++	Male
—	2	32	+++	+++	—
528	3	20	+++	+++	Female
—	4	28	+++	+++	Male
—	5	28	+++	+++	—
—	6	28	+++	+++	—
—	7	28	+++	—	Female
—	8	29	+++	+++	Male
—	9	29	+++	+++	—

(3) Wild Fly Strain E.

Table VIII gives the transmission experiments, Table IX the results of the fly dissections with this strain.

Table VIII.

Experiment No.	Infecting monkey	Date	Flies dissected			Day on which dissection began	Alive 25th day	Date at which box became infective	Flies containing flagellates	Result of feeding on clean monkey	Duration of experiment
			Males	Females	Total						
513	491	16, 17, 18.9.22	21	19	40	12	34	—	0	—	44
526	491	21–23.9.22	32	39	71	8	62	22	23	+	35
531	492	23–25.9.22	41	23	64	9	54		7		33
540	491	26–28.9.22	40	18	58	25	56	23	3	+	34
542	492	27–29.9.22	30	43	73	8	55		5		34
544	489	28, 29, 30.9 and 1 and 3.10.22	28	23	51	7	44	22	4	+	34
545	489	28.9–1.10.22	34	29	63	8	53		0		33
547	492	30.9–2.10.22	22	26	48	8	37	26	1		32
548	491	2.10–4.10.22	32	35	67	6	52		3	+	29

The brackets indicate that the two boxes were fed upon the same clean monkey on alternate days.

TABLE IX.

Experiment No.	No. of fly containing flagellates	Day of experiment on which fly was dissected	Intensity of infection		Sex of fly
			Gut	Glands	
526	1	10	+++	(not seen)	Female
—	2	35	++	—	Male
531	3	14	+++	—	Female
—	4	18	+++	—	—
—	5	33	+++	—	Male
—	6	33	+++	+++	Female
—	7	33	+++	+++	Male
—	8	33	+++	+++	—
—	9	33	+++	+++	—
540	10	33	+++	+++	—
—	11	32	+++	+++	—
—	12	32	+++	+++	Female
542	13	17	+++	—	Male
—	14	20	+++	—	Female
—	15	34	+++	+++	—
—	16	34	+++	+++	—
—	17	34	+++	+++	—
544	18	20	—	—	Male
—	19	33	+++	—	—
—	20	34	+++	+++	—
—	21	34	—	—	—
547	22	20	+++	—	Female
548	23	26	+++	+	Male
—	24	29	+++	+	—
—	25	29	+++	+	Female

It will be seen from Tables IV-IX that all the three strains were readily transmissible by *G. palpalis* at the time of their isolation from the fly-belt.

The experiments arrayed in Table X were carried out in March and April, 1924, with the only strain surviving at that time, Human Strain 483.

TABLE X.

Experiment No.	Infecting monkey	Day on which dissection of flies began	No. of flies alive on 20th day	Flies dissected			Duration of experiment days	Day of disease in infecting monkey when flies put on
				Males	Females	Total		
1	837	14	45	27	22	49	31	36–38
2	848	29	50	24	26	50	31	7–9
3	837	9	47	24	27	51	30	39–41
4	848	11	55	29	30	59	32	10–12
5	849	22	62	33	29	62	32	11–13
6	837	9	65	42	30	72	33	42–44
7	848	8	50	25	29	54	33	13–15
8	849	9	68	35	34	69	36	14–16
9	837	8	43	44	12	56	37	45–47
10	849	11	54	30	29	59	36	17–19
11	848	10	51	38	23	61	39	17–19
12	837	6	56	36	27	63	38	49–51
13	848	8	57	34	33	67	38	20–22
14	849	9	41	33	26	59	38	21–23
15	837	8	32	25	27	52	42	52–54
16	848	11	57	34	27	61	41	23–25
17	837	11	47	36	23	59	35	55–57
18	848	9	56	38	29	67	35	26–28
19	849	8	47	26	33	59	37	28–31
20	837	11	56	34	26	60	38	58–60
21	837	9	50	36	19	55	36	61–63
22	849	9	30	24	22	46	37	31–33
23	849	10	58	37	30	67	38	32–34
24	837	10	60	29	34	63	38	63–65
25	849	12	31	15	19	34	37	35–37
26	837	9	19	12	16	28	38	65–67
	Totals	... ...	1287	800	682	1482		

Of the 1,482 flies dissected during these experiments, six were found to contain flagellates. In every case, however, the organisms were exceedingly scarce, amounting to a few individuals in the whole preparation of the teased-out gut. The particulars of these six flies are as follows :—

1. *Experiment* 2 : One female killed on thirty-first day after first infective feed.
2. *Experiment* 6 : One eleventh day male.
3. *Experiment* 8 : One ninth day male.

4 and 5. *Experiment* 9 : One tenth day male, and one thirty-fifth day male.

6. *Experiment* 10 : One twenty-second day male.

The minute infections shown by these flies were very remarkable. They were only revealed by a careful search of the freshly-teased gut, and sometimes only two or three flagellates were seen. They must be regarded as abortive attempts at development by a strain which has all but completely lost its power of maintaining itself in the alimentary canal of *Glossina*. When a normal fly-strain is exposed to tsetse the trypanosomes die out at once in the majority of the flies ; but, in the favourable minority, development proceeds uninterruptedly, until the gut of the insect is swarming with flagellates.

The condition in these six flies is quite different from anything hitherto observed, and represents a still further stage in the degeneration process already demonstrated in earlier work done with the Antelope Strain (Duke, 1923b).

PART II

A.—RESUMÉ OF EXPERIMENTAL WORK ON DIRECT TRANSMISSION OF TRYPANOSOMES

The experiments set forth in this paper complete a series of three parallel studies on the effect of continued direct transmission on polymorphic trypanosomes of the *brucei* group. The strains examined are the Direct Transmission Strain, isolated from wild Lake-shore *G. palpalis* in January, 1920; the Antelope Strain, isolated from a situtunga antelope on Damba Island in September, 1920; and the Mwanza Human Strain, 483 (Duke 1921, 1923a and 1924).

All these strains, when first isolated, were readily transmissible cyclically by laboratory-bred *G. palpalis* at the Entebbe Laboratory. A comparison of the histories of the three strains since their arrival at the Laboratory is interesting.

(1) Direct Transmission Strain.

After 11 direct passages, covering 16 months, was still cyclically transmissible by tsetse.

After 23 more passages, over a further period of 14 months, was no longer able to develop at all in tsetse. The exact period at which this change set in was not determined. A sudden increase of virulence appeared about the eighteenth passage, after 23 months' maintenance by direct transmission. This increase in virulence apparently occurred at about the time that the trypanosomes lost their power of developing in tsetse.

(2) Antelope Strain.

After 24 direct passages, over a period of about 22 months, had lost the power of invading the salivary glands of the fly; heavy gut-infections were still obtained, but here also there were signs of impairment in developing power. Ten months later, after 20 more passages, all power of development in tsetse had disappeared. The exact date at which this last stage was reached was not determined.

The virulence of this strain, in monkeys, apparently began to increase about the seventeenth direct passage, *i.e.* after about

15 months of maintenance. The average duration of the disease in the 24 monkeys employed during the first 16 direct passages was 34 days; during the subsequent 34 passages, extending over a period of 20 months, the average duration for 20 monkeys was 24 days.

It is impossible to determine with certainty if the virulence began to increase coincidently with the onset of impairment in the power of cyclical development in the fly. By the time the trypanosome had lost its hold on the salivary glands of the fly, virulence had begun to increase, and it may well be that the two processes were intimately associated.

(3) Mwanza Human Strain.

Table V suggests that the strain had already undergone slight modification in relation to tsetse towards the end of its sojourn in monkey 469, *i.e.* during the second direct passage from man, and only two months after isolation. This inference is, however, by no means certain, as the baboon's blood may have influenced the development of the flagellates. The passage to the next monkey of the series was made by flies cyclically infected from 469. Eleven months later, after eight direct passages, the strain had all but completely lost the power of development in tsetse. The virulence of this strain in monkeys showed no increase at the time that the last transmission experiments (shown in Table X) were carried out. But in the passages immediately following, an increase was at length manifest, and the strain has been now transferred to guinea-pigs.

It will be noted that the Mwanza Strain lost the power of cyclical development in tsetse much more rapidly than did the two Uganda strains. Both the latter were undoubtedly cyclically transmitted previous to their isolation in monkeys. On the other hand, there are reasons for suspecting that Strain 483 may have been subjected to a number of direct passages from man to man in the fly-belt before reaching native S, and this may explain the relatively rapid alteration of this strain after its arrival at the laboratory.

B.—DISCUSSION

The results obtained from these transmission experiments, though of scientific interest, are not necessarily valid for human trypanosomiasis. Monkeys cannot be regarded as true natural hosts of the trypanosome ; and propagation by the syringe differs in many respects from any natural method of transmission. We are, indeed, witnessing the behaviour of what is practically a *monkey strain* of a trypanosome under peculiar conditions of maintenance, and we have recorded the appearance of certain alterations in the physiology of the strain. But caution is necessary in attempting to assign a cause for these changes, lest we ascribe to the agency of direct transmission effects due wholly, or partially, to the tissue reactions of the *Cercopithecus*. Not that there is any reason to suppose that the host factor alone, without the co-operation of direct transmission, can account for the loss of the power of a strain of trypanosomes to develop cyclically in tsetse. Provided that the passage of the monkey-strain from animal to animal is always effected by cyclically infected flies, it is a reasonable, though unproven, assumption that the parasite will retain indefinitely its power to infect the salivary glands of the fly. Presumably, too, any departure from the cyclical method will tend, sooner or later, to alter the constitution of the trypanosome ; though in the case of the Direct Transmission Strain (Duke 1921), the organism was still capable of full cyclical development in *Glossina* after eleven monkey-passages by the direct method over a period of some nine months.

On biological grounds we should expect that cyclical transmission, acting over a prolonged period, would lead to a gradual adjustment of the trypanosome to its mammalian host. An excellent example of this is the equilibrium established between ruminant game and *T. brucei*. In nature, this adjustment will be found to exist between a trypanosome and those species of animals upon which it is normally dependent. In certain *palpalis* regions there is evidence that a balance has been established between man and a member of the polymorphic group of trypanosomes. But in most, if not all, human communities where trypanosomiasis occurs, man is evidently too susceptible to qualify as an ideal host ; and while we have no evidence that game animals in their wild state are inconvenienced by their trypanosomes, in the case of man the difficulty is rather to prove the existence of any genuine tolerance.

Vertebrates in which the trypanosome provokes a rapidly fatal disease cannot be regarded as biologically perfect hosts of the parasite. Monkeys fall into this category, and so, to a great extent, does man. The strains which we have been studying in monkeys were maintained in an artificial environment. They destroyed their hosts rapidly; could not develop in tsetse; and were absolutely dependent on direct transmission for survival. The Mwanza human trypanosomes were, as a rule, rapidly fatal to their human hosts, though retaining (at the time of the first sub-inoculations into monkeys) the power to survive in tsetse; and there are reasons for believing that direct transmission played an important part in the spread of the Mwanza disease in man. To a certain extent, therefore, the monkey-strains under review are comparable with the virulent human strains in the fly-belt, *i.e.* they are both utilising vertebrate hosts to which they are imperfectly adjusted biologically. The closeness of the parallel will, of course, depend upon the extent to which vertebrates other than man are accessible to the trypanosome; in other words, to the frequency with which direct transmission from man to man is effected. In all probability, therefore, similar causes will produce similar effects, and the evolution of these virulent human strains in the fly-belt, if left to nature, will be subject to the laws that govern the behaviour of our monkey strains at the laboratory.

The *brucei-gambiense* group of trypanosomes is widely associated in nature with certain species of ruminant game. The organisms are transmitted by cyclical passage through the *Glossina* that feed upon these animals, and, apparently, no ill-effects are caused to the mammalian host. When circumstances compel a trypanosome to resort to species of mammals that possess little or no tolerance towards it, cyclical transmission by *Glossina* will tend to establish equilibrium between the parasite and its vertebrate host. If the mammal be unable to tolerate the infection and equilibrium is unattainable, the trypanosome may gradually lose its power of cyclical development, until it becomes entirely dependent on direct transmission for its propagation. For a time, however, even in highly susceptible hosts, cyclical transmission will still function, and will, no doubt, tend to delay the appearance in the strains so transmitted of the fulminant characters so favourable to the direct method.

We know of the existence in nature of three free-flagellated mammalian trypanosomes which have dispensed with cyclical

transmission—*T. evansi*, *T. equinum* and *T. equiperdum*. As far as is known, all these organisms rely for their propagation entirely on direct transference from host to host. Surra, mal de caderas and dourine are generally recognisable by the clinical symptoms they produce. But apart from the recognition of these organisms on clinical or morphological grounds, it is a somewhat tedious process to demonstrate the inability of a trypanosome to develop cyclically in *Glossina*, and this test is not often applied to strains isolated in the field. I do not know whether posterior-nuclear forms occur in animals infected with surra ; but, in any case, the recovery from game or stock of a virulent trypanosome of the *brucei* group, incapable of cyclical development in tsetse, would inevitably suggest thoughts of *T. evansi*. It may well be that these three trypanosomes have evolved, in the manner outlined above, from fully-equipped tsetse strains of the *brucei* type.

Unfortunately for our present investigation, man is distinguished among the mammals commonly fed upon by *Glossina*, in that his relation to the trypanosomes of the *brucei-gambiense* group ranges from complete immunity to acute susceptibility. The baboon, which is exceedingly resistant to all trypanosomes, has never been found naturally infected, although it has, on rare occasions, been infected in the laboratory. This animal cannot, therefore, be made to take the place of man in experimental investigations of the polymorphic group. As far as is known, the chimpanzee is susceptible to all the trypanosomes of this group. It is man's unique position with regard to trypanosomes that makes the problems of human trypanosomiasis so difficult to solve. In *palpalis* areas, the conclusions resulting from our laboratory investigations of monkey-strains can be applied intelligibly to the study of the trypanosomes in man. Both endemic and epidemic types of the human disease are known to occur, and it is conceivable that direct transmission strains, incapable of cyclical development in tsetse, do actually exist in certain human communities. But in game-tsetse areas the case is different and more difficult to unravel. Man is very much more resistant to the trypanosomes of the *brucei-gambiense* group than are most of the species of animals upon which tsetse feed. In the case of cattle, for example, it is well known that the briefest exposure of the animals in a fly-belt results in their becoming infected ; but numbers of

natives have lived for years in contact with game-tsetses without mishap. Apparently the great majority, if not all, of the cyclically-carried wild-fly strains of polymorphic trypanosomes in game-tsetse areas are innocuous to healthy man. Yet there exists, in some of these areas, a certain number of human beings who carry in their blood trypanosomes indistinguishable from the ubiquitous game organism. An attempt has been made elsewhere to discuss the etiology of these human strains (Duke, 1923b). The German view is that the human parasite, the so-called *T. rhodesiense*, is specifically different from *T. brucei*. The ability to infect man is regarded as possessing the importance of a specific character, and therefore, presumably, sufficiently deep-rooted to survive cyclical passage through tsetse. Now there is no evidence, as far as I know, to warrant this assumption. If such a genuine human parasite existed, regularly transmissible from man to man cyclically by *Glossina*, we should expect to find a considerably larger number of cases of human trypanosomiasis than actually occur in the populations of infected *morsitans* areas. We have no evidence of the existence in man in these game-tsetse areas of a mild form of trypanosomiasis analogous to that found in game animals.

There is obviously a hitch somewhere in the progress of the trypanosome as a human parasite in these regions. Its behaviour in man and the curious distribution of the disease are not adequately explained by the theory that the parasite relies mainly, if not entirely, on cyclical transmission for its passage from man to man. On the other hand, several facts point to the intervention of direct transmission. The human host succumbs to a fatal disease, in the course of which the peripheral blood is heavily charged with trypanosomes. The clinical condition is thus favourable to direct transmission, and this is further facilitated by man's gregarious habits and his hairless skin. By direct transference to another human host the trypanosome evades the ordeal of cyclical passage through the tsetse, and the attendant risk of losing its precarious hold on man.

We have seen above that a strain may undergo several passages by direct transmission without impairment of its transmissibility by the cyclical method. Possibly this observation has an important bearing on the problem before us. A series of direct passages from man to man may confer on a strain of trypanosomes, attributes

sufficiently deep-rooted to survive cyclical passage through *Glossina*. In this way more or less stable cyclical human strains might arise in the wake of an epidemic.

Direct transmission offers an explanation of the sporadic distribution of the human disease in certain *morsitans* areas. In the presence of tsetse, a sick man whose blood is swarming with trypanosomes will afford excellent opportunities for the direct transference of his blood, by the fly, to his immediate neighbours ; whereas those of his trypanosomes which succeed in establishing themselves cyclically in the tsetse may, on the completion of their development in the fly, be no longer able to survive in man's blood.

There is a widespread tendency to assume that all human cases derive their infection from cyclically-infected wild flies. Great stress is laid on the fact that so-and-so became infected shortly after visiting a fly-area to hunt ; but the facts that nearly every male in the area is a hunter, and that the patient himself or his friends and relations live in more or less close contact with tsetse, are apt to be overlooked.

In such a community, parties of natives will visit the uninhabited fly-country from time to time to hunt, or to cut poles, or for some other objective, and there is nothing to prevent an early case of trypanosomiasis from participating in these excursions. In the presence of hungry tsetse such a man is a menace to his fellows. One can hardly imagine conditions more ideal for the distribution of the sick man's trypanosomes by the direct method than those obtaining during the later stages of a native hunt in *morsitans* country. I believe that a ' clean ' native entering one of the so-called infected *morsitans* areas is much more likely to be infected by the fly if one or more of his companions are already suffering from trypanosomiasis, than if he goes into the fly-country alone or with ' clean ' companions. Until we have irrefutable evidence that ' clean ' natives, from a ' clean ' area where there is no fly and no human trypanosomiasis, can contract the disease by entering a fly-area where there are no people, we should at least maintain an open mind about the relative importance of cyclical and direct transmission in the spread of human trypanosomiasis in game-tsetse areas.

In the present state of our knowledge we may suppose that,

under circumstances which are not yet understood, the game trypanosome, *T. brucei*, can establish itself in man, using him as an adventitious host and causing a fatal disease, a feature of which is a heavy infestation of the peripheral blood. The parasite thus stands an excellent chance of being transmitted mechanically to fresh human hosts, and of maintaining intact those qualities, whatever they may be, which originally determined its establishment in man.

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EXPERIMENTAL STUDY OF TRYPANOSOMIASIS IN PALESTINE

BY

I. J. KLIGLER

AND

I. WEITZMAN

(From the Laboratory of the Malaria Research Unit, Department of Health, Haifa)

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I. EXPERIMENTAL TRANSMISSION, SYMPTOMS AND PATHOLOGY

There is little information extant regarding the prevalence of trypanosomiasis in Palestine. There are no records of its existence prior to the war, but during the war trypanosomes were reported to have been found in army camels. Goldberg (1917) reports a severe epidemic in 1915, among the camels of the Turkish Army, during which 462 out of 956 camels died. The author classes the trypanosomes as *T. evansi*. Stuart (1923) reports that he found trypanosomes in a number of sick camels belonging to the army of occupation, which he also identified as *T. evansi*. There is, however, no record of this disease in native horses and mules.

Recently Dr. Deuel, a local veterinary surgeon discovered an outbreak of trypanosomes in mules on a farm in Northern Palestine. The nature of the disease was, apparently, not clear, since the veterinarians differed with regard to its character ; some claimed it was Nagana, while others classed it as Dourine ; Surra was, apparently, not suspected. In order to clear up this matter we undertook a study of the disease.

History of the outbreak. In September, 1922, an English mare and a native mule became ill with what appeared to be haemoglobinuria, but laboratory examinations established the presence of trypanosomes in the blood. In January, 1923, three more mules became ill and shortly after another one, making a total of 6 out of 15 animals. The symptoms were : emaciation, swelling of the breasts, intermittent fever, and occasionally haemoglobinuria. The diagnosis of trypanosomiasis was made and confirmed by blood films examined in the Hadassa Laboratory at Saffed, and by Dr. Stuart in the Central Government Laboratory. Three of the mules were native animals and two were imported from Syria ; the mare was bought in Saffed, but its past history was not known. All the animals, but the mare, had been on the farm two or three years prior to the outbreak of the illness ; the mare was the only animal acquired about four months before the discovery of the illness.

Transmission of the Infection. Thanks to the assistance of the Chief Veterinarian of the Department of Agriculture, we obtained two of the sick mules, one male and one female, for our studies.

The animals were brought to the laboratory yard and kept there for observation.

At the time of their arrival neither of the animals had trypanosomes in the blood. Nevertheless, blood was inoculated into two dogs and successful transmission obtained after an incubation period of seven days.

Subsequent to the first transmission experiment and prior to the appearance of parasites in the blood of the infected animals, we were advised by a local veterinarian (Dr. Freund) to try the effect of arecolin on the mobilization of trypanosomes in the peripheral circulation. The results were quite interesting. A dose of 0·05 gm. injected subcutaneously caused, almost immediately, marked salivation, defecation and urination. Forty-five minutes after the injection the blood was still negative, but after one hour trypanosomes were present in the peripheral blood. The drug had the identical effect in both mules.

After the appearance of trypanosomes in the blood, a second series of transmission experiments was made from both mules into dogs, rabbits and guinea-pigs. The blood was taken with a syringe from the jugular vein, a portion of it was mixed with 1·5 per cent. citrate solution, and varying amounts of the whole and citrated blood were inoculated intraperitoneally into animals as indicated in the table below. All of the animals showed trypanosomes in the blood after an incubation of six to eight days, with the exception of one guinea-pig in which the incubation period was eleven days.

TABLE I

Transmission of Trypanosomes from Infected Mules to Lower Animals

	Mule 1 (black, male)		Incubation	Mule 2 (white, female)		Incubation
	No.	Dose		No.	Dose	
Dogs	1	5 c.c. whole blood	6 days	1	5 c.c. citrated blood	7 days
				1	10 c.c. „ „	6 „
Rabbits ...	1	3 c.c. „ „	7 „	1	3 c.c. „ „	7 „
Guinea-pigs ...	2	2 c.c. „ „	7 & 8 „	2	2 c.c. „ „	8 & 11 „

Subsequent transmissions were carried on from guinea-pigs and rabbits. As a rule the virus was passed on in the same animal species : but occasionally transfers were made from rabbits to guinea-pigs and *vice-versa*. Inoculations were always made intraperitoneally with either whole or citrated blood. The strain has now been carried over a year through many generations and the disease has been observed in two mules, three dogs, and a large number of rabbits and guinea-pigs. It is, therefore, possible to give a composite picture of the experimental disease as it manifests itself in these animal species.

Incubation. In dogs the incubation period varied between five and seven days, average six days ; in rabbits between six and eight days, average seven days ; in guinea-pigs between four and eleven days, average seven days. These incubation periods correspond in general with those obtained by other observers with strains of *T. evansi* (Brown and Pearce, 1918).

The presence of parasites in the peripheral circulation was very irregular. In all the animals there were periodic remissions of greater or lesser duration, but without any regularity. Parasites were always more abundant in guinea-pigs and dogs than in rabbits. In the former they became particularly abundant a short time before death, while in rabbits the reverse was usually the case. Parasites were never abundant in the blood of the rabbit, and they were practically always absent before death.

Duration. The infection was invariably fatal for all animals studied, but the duration of the disease varied with different species. The duration in the mules is not known, but it was longer than six months. One dog succumbed after an illness of one-and-a-half months ; the other two were treated and cured. Rabbits died usually within five to eight weeks after the appearance of parasites in the blood ; young rabbits, however, weighing 700 to 800 grams, succumbed in three to four weeks. In guinea-pigs the results were anomalous—at the beginning they died after four to six weeks, but in the later passages the virulence seemed to have diminished and the animals survived twelve weeks or longer. There was apparently a decrease in virulence due to animal passage.

Clinical Symptoms. The two spontaneously infected mules which were sent to us were anaemic and emaciated. They were

weak, listless and unable to work. An intermittent oedema of the breasts and legs, were observed, but no abnormalities in the genitalia. There was intermittent fever and occasionally a mild haemoglobinuria.

In the experimental animals the clinical picture varied with the animal used. Dogs showed the effect of the infection after two weeks. They became listless, lost their appetite and had an appearance of being very ill. Guinea-pigs, on the other hand, showed hardly any signs of illness, and even when there were large numbers of trypanosomes in the blood the animals showed no loss of appetite. Only in rare cases of long standing infection a few skin lesions were noticed. In rabbits the symptoms were more striking, but the signs of the illness appeared more slowly than in dogs. The common features of the infection in all the animals were : an intermittent temperature, with appearance of parasites in the circulation during the febrile period ; a lymphocytosis, particularly shortly before and during the onset of fever ; abnormal changes in the red cells. In the rabbit and dog conjunctivitis and keratitis were constant signs, while in the guinea-pig they were rarely observed.

Blood Picture. The blood of infected animals showed typical changes. During the incubation period the blood picture and blood count remained normal, with the exception of a slight leucocytosis which occurred on the first day after the inoculation. With the appearance of signs of the infection, sometimes even one or two days before parasites were found in the circulation, there developed a leucopaenia associated with a definite lymphocytosis. At the same time there were changes in the red blood cells characteristic of progressive anaemia : polychromatosis, anysocytosis, large 'crescents' and Cabot's rings, which persisted, with some variations, throughout the course of the infection. The accompanying table (p. 442) is typical of the blood changes observed.

Pathology. The macroscopic and microscopic pictures of the disease varied with the animals used. In general, however, the infection in the dog and older rabbits resembled more closely that of the spontaneously infected mules than did the disease in guinea-pigs. In the rabbit and dog, as in the mule, the course of the disease was more chronic and there was extensive subcutaneous oedema and perivascular round cell infiltration in the various organs. In

the guinea-pig, the course of the disease was more acute and toxic in character ; often the guinea-pig died from the infection, without showing any external signs whatever, other than progressive loss of weight.

NATURE OF THE DISEASE. The evidence presented by our observations on the naturally and experimentally infected animals leads us to believe that we are dealing with Surra, and that the trypanosomes responsible for the condition belong to the *T. evansi* group. In morphology the organisms belong to the monomorphic group. They are slender, actively motile trypanosomes, 18 to 22μ long and 1·5 to 2·0μ wide. The posterior extremity is slightly pointed ; the undulating membrane is well-defined ; the flagellum

TABLE II

Blood Picture in Experimental Trypanosomiasis in Guinea-Pigs
(Inoculated October 22, 1923)

	23	24	25	26	27	28	29	30
W.B.C.	9,600	10,000	8,400	6,000	5,200	4,800	5,600	5,600
Polynuclears (%) ...	69	67	50	42	37	32	37	40
Lymphocytes (%) ...	28	30	46	55	59	63	59	55
Large mononuclears and transitionals (%) ...	3	3	4	3	4	5	4	5
Blood picture	normal	normal	anaemic 'crescents'	polychromatosis, 'crescents,' normoblasts.				
Trypanosomes	−	−	−	−	+	+	+	+

is free and fairly long—about one-third or less the length of the parasite. The nucleus is large, oval and central ; the blepharoblast is round and situated about one-quarter to one-third of the distance between the posterior end and the nucleus. The protoplasm is uniform or slightly granular.

The incubation period in experimental animals of five to seven days, the pathogenicity for the dog, rabbit and guinea-pig, the inability to transmit the infection by coitus and the picture of the spontaneous and experimental disease correspond fairly closely with that observed by other investigators in Surra.

CULTIVATION OF THE TRYPANOSOMES. Cultivation experiments

thus far have failed completely. A large variety of media have been tried, including the classic N.N.N. medium, the medium used by one of us (Kligler, 1924) in the cultivation of Leishmania, Noguchi's media and various modifications ; but the results have thus far been uniformly negative. The only result obtained was survival of trypanosomes for three days in normal rabbit serum incubated at 22° to 26° C.

II. EFFECT OF 'BAYER 205' IN EXPERIMENTAL TRYPANOSOMIASIS

Curative Power of the Drug. In our experiments we used dogs, rabbits, and guinea-pigs. Although mice are often preferred in chemo-therapeutic experiments on account of the ease with which parasites are found in the blood, the disease in the rabbit and the guinea-pig resembles more closely that in the mule than does the infection in mice ; the former are, therefore, more satisfactory for therapeutic tests. We also found that, using the thick drop method, it was not at all difficult to find the trypanosomes in the blood of rabbits and guinea pigs even if present only in small numbers. The blood picture in these animals is another valuable aid in following the course of the infection ; in the guinea-pig, at least, we have found it almost as diagnostic as the presence of parasites. Leucopaenia and anaemic elements (Crescents, Cabot's rings, etc.) are constant throughout infection, but disappear when the animal is cured. It is possible, therefore, by the combined method of thick drop examination and a study of cellular elements of the blood to follow without difficulty the course of the infection and the effect of the drug.

In testing the therapeutic value of the drug, we tried to determine the minimum single dose which will uniformly sterilise infected animals. The doses varied from 0·2 gm. to 0·04 gm. per kilo of body weight. The injections were made intraperitoneally from 5 per cent. or 10 per cent. solutions of the drug.

In the course of these experiments we treated a large series of guinea-pigs and rabbits in various stages of the illness, with varying amounts of the drug. Doses of 0·1 gm. per kilo or over sterilized all rabbits and guinea-pigs without exception. A dose of 0·05 gm. per kilo of body weight did not yield 100 per cent. of cures. Of fifteen

guinea-pigs treated with this dose two relapsed, one three days and the other fourteen days after treatment. Similarly one of five rabbits treated with this dose of the drug relapsed after eleven days. In all cases of relapses the condition of the animal rather than the duration of the illness seemed to be the important element. The two guinea-pigs had received the infection twenty-four days and the rabbit twenty days before treatment ; other animals treated at the same stage of the infection recovered completely.

It is evident from these experiments that a single dose of ' Bayer 205 ' is capable of sterilizing the blood of dogs, rabbits and guinea-pigs infected with a virulent strain of trypanosomes (*T. evansi*) with the apparent production of a permanent cure. The drug was administered at various stages of the infection, always with the same results.

Animals in the later stages of the infection, showing definite physical signs of the disease at the time of treatment, recovered completely and these signs cleared up within a few days after the drug was administered. Two of the guinea-pigs had advanced skin lesions at the time of treatment and within a few days the ulceration ceased and the wounds healed progressively. The smaller doses of the drug (0·05 gm. per kilo) were more satisfactory, despite the fact that a few of the animals relapsed, because of the practical absence of any noticeable toxic effect. The large doses gave more uniform results, but, as will be noted below, they were not far removed from the toxic dose of the drug, and often they actually produced toxic symptoms.

Toxic Effect of 'Bayer 205.'—In the course of our experiments, we have had occasion to note that the drug is highly toxic for rabbits and guinea-pigs. Several of our animals which were treated with the larger doses (0·1 to 0·2 gm. per kilo) died within a short period after the administration of the drug without any apparent clinical cause. Two rabbits died within six and twelve days and three guinea-pigs within two, six and seven days, respectively, after the injection of the drug. We, therefore, injected various doses of the drug into a series of animals in order to determine the lethal dose of the drug and the pathological changes produced in the body. A dose of 0·4 gm. per kilo of body weight injected intraperitoneally killed a medium size guinea-pig (460 gms.) in four days. Smaller

doses are not uniformly lethal ; a guinea-pig which received 0·25 gm. of the drug per kilo died seventeen days after the injection with typical pathological changes, while at the same time another guinea-pig receiving 0·3 gm. per kilo remained alive and well. These tests show that, for the guinea-pig at least, the therapeutic dose is approximately one-fifth to one-eighth of the lethal dose. But even smaller doses (0·1 to 0·2 gm. per kilo), which may have little effect on normal guinea-pigs or rabbits, may be decidedly toxic when injected into animals infected with trypanosomes.

Pathological Changes produced by the Drug.—In the infected animals it was difficult to differentiate the changes due to the infection from those caused by the drug. The infected animals which died after treatment showed a greater or lesser degree of degeneration of the kidney tubules, but this type of lesion was also characteristic of animals which died from a trypanosome infection. The changes found in the control guinea-pigs may be accepted as characteristic of the lesions produced by the drug. The principal lesion was extensive degeneration of the cells of the tubules, chiefly in the cortical region. The blood vessels were also congested. Another striking feature was a deposition of a brown pigment in the spleen. The liver was congested and there were, here and there, small areas of necrosis, but the changes were not as striking as those in the kidneys. The lesions found in the kidney taken with the reported presence of albuminuria in patients treated with ' Bayer 205 ' suggest a selected toxic affinity of the drug for the kidney tubules. Recently Duncan and Manson-Bahr (1924) reported almost identical changes in mice injected with lethal doses of the drug.

Prophylactic Effect of ' Bayer 205.'—Mayer and Zeiss (1921) emphasize the fact that ' Bayer 205 ' remains a long time in the animal body and consequently serves as a prophylactic against infection. Brumpt (1924) has made similar observations. These authors worked with mice. Kleine (1924) working with larger animals, failed to get protection against infection. We have tested the prophylactic effect of various doses of the drug in rabbits and guinea-pigs. The following are typical protocols :—

Rabbit Y. Weight 1390 gms. received on 1.7.23, intraperitoneally 0·10 gm. ' Bayer 205 ' per kilo of body weight. On 1.8.24, one month later, the rabbit received an infective dose of trypanosome blood. The blood of the rabbit remained negative. One month later this rabbit received another dose of infected blood,

and after an incubation period of 10 days, trypanosomes appeared in the circulation.

Rabbit 17. Weight 1680 gms., 0·05 gm. per kilo 'Bayer 205' given intraperitoneally, 16.12.23. This was followed after 25 days by an injection of trypanosomes. The result was negative. Sixty-three days later another infective dose given; after 15 and 18 days trypanosomes were found in the blood; since then the blood remained negative, but the animal developed all the clinical signs of chronic trypanosomiasis.

Rabbit 15. In this case the infective dose was given three days before the injection of 0·05 gm. 'Bayer 205' per kilo. Twelve days after the infection trypanosomes appeared in the circulation for one day. After that the blood remained negative, although clinical symptoms developed. Five weeks after the first appearance of trypanosomes the parasites were again found in the blood, and they persisted intermittently until the death of the rabbit.

Guinea-pig X. Weight 440 gms., received 0·2 gm. 'Bayer 205' per kilo, intraperitoneally and one month later an infective dose of trypanosomes. After a somewhat prolonged incubation period of 15 days, trypanosomes appeared in the circulation.

Guinea-pig 34. Weight 440 gms., 0·3 gm. 'Bayer 205' per kilo injected intraperitoneally on 16.11.23. After 25 days an infective dose of trypanosomes inoculated intraperitoneally. Trypanosomes found once after 36 days and thereafter the animal was normal. After 88 days the animal was reinfected a second time, but no signs of infection developed during the month following the infection. The animal died from a bile injection.

These observations indicate that the drug affords a considerable protection, and that the degree and duration of the protection varies with the amount of drug administered and the individual animal. It is noteworthy that the drug given shortly after the infection, that is, during the incubation period, did not prevent the development of the disease.

Trypanocidal Action of 'Bayer 205' in vitro.—Tests made *in vitro* to determine the trypanocidal action of this drug gave totally different results from those obtained *in vivo*. In the test tube a concentration of 1 : 100 of the drug failed to immobilize this strain of trypanosome in five to eight hours; while in the animal body 0·05 gm. per kilo (a dilution of 1 : 2000 or less) sterilized the blood of infected animals in fifteen hours.

Several modified procedures were employed in these experiments. In one method blood was drawn from an infected animal into a test tube containing a few glass beads. The blood was defibrinated and divided into small Wasserman tubes : 0·9 c.c. into the first tube and 0·5 c.c. into four or five others. 0·1 c.c. of a 10 per cent. solution of 'Bayer 205' was added to the first tube and further dilutions made by adding 0·5 c.c. from tube 1 to tube 2, etc. This gave a series

of dilution of 100, 200, 400, etc. The tubes were kept at 25° C. and drops examined under the dark field microscope at various intervals. This was the method of choice, because the trypanosomes remained in a favourable medium and the conditions approximated those in the animal body.

Another method consisted in the use of infected citrated plasma from which the red cells had been removed by centrifugalization for five to six minutes at 500 revolutions per second. By slow speed centrifugation the red cells are removed, while a sufficiently large number of actively motile trypanosomes remain in the supernatent plasma for the purpose of the experiments. The rest of the procedure was the same as with the defibrinated blood.

A third method consisted in the use of normal rabbit or guinea-pig serum, containing various dilutions of the drug to which small amounts of infected blood or serum were added.

Whichever method was employed the results were the same. During the first four to eight hours no effect was noted on the motility of the trypanosomes in dilutions of 1 : 100 or more. After twenty-four hours, active trypanosomes were still encountered in the 1 : 100 dilution, but the number was considerably less than in the control tubes. After forty-eight hours no motile trypanosomes were found in the dilution of 100 and 200, but the higher dilutions still contained actively motile trypanosomes.

Similar observations were made with bacteria. Contaminating air bacteria as well as *B. coli* multiplied actively in tubes containing 1 : 100 dilution of the drug.

It is apparent from these results that under the conditions of the experiments the drug is only slightly trypanocidal. This fact is of no particular importance in so far as the therapeutic value of the drug is concerned. It is of interest, however, in relation to the mechanism of the action of the drug. It is clear that either ' Bayer 205 ' undergoes some change in the body which renders it an effective trypanocide, or that it acts indirectly on the trypanosomes by inducing certain changes in the resistance of the host. In this connection it is noteworthy that the injection of ' Bayer 205 ' is followed by a marked increase in the total leucocyte count as well as in the large mononuclears ; in some animals this increase is very large and persistent.

III. MECHANISM OF RESISTANCE TO TRYPANOSOME INFECTION

Resistance of Cured Animals to Reinfection.—It is well-known that rats which recover spontaneously from an infection with *T. lewisi* are immune to a reinfection with that organism. It remained to determine whether recovery from an infection with a pathogenic trypanosome will also confer immunity on these animals. We realised that the analogy was not absolute, since our animals did not overcome the infection naturally as in the case of the rats. Nevertheless, we investigated this point by attempting to reinfect guinea-pigs and rabbits cured with 'Bayer 205.'

At various intervals after treatment with 'Bayer 205' the cured animals were injected with infective blood. These reinfections were repeated at various intervals under different conditions. All of the animals, without exception, showed a marked degree of resistance to reinfection, although the duration of this resistance varied. The resistance of guinea-pigs was apparently more persistent than that of rabbits. Several of the animals died after the second or third attempt at reinfection, but none of them showed any clinical signs of infection at the time. Reinfections were obtained in three rabbits out of the entire series and in these cases the resistance was apparently broken down artificially.

Table III (p. 449) gives a summary of the reinfection experiments, showing the duration and degree of the resistance in the different animals.

It is apparent from these results that infected guinea-pigs and rabbits cured with 'Bayer 205' develop a resistance to reinfection of long duration. In rabbits this resistance lasts three to four months, while in guinea-pigs it persists for longer periods.

Nature of Resistance.—The experiments recorded in Section 2 indicate that 'Bayer 205' injected into normal animals protects guinea-pigs and rabbits for a greater or lesser period, depending on the amount of the drug injected. This protection was not, however, as lasting as that observed in infected animals cured with 'Bayer 205.' In cured animals long-standing resistance develops even when treated with so small a dose as 0·05 gm. per kilo of body weight. Kleine and Fischer (1922), working with monkeys, also found that

cured animals developed a more persistent resistance to reinfection than those given a prophylactic dose of the drug.

In view of the bearing that this phenomenon may have on the general problem of immunity or resistance to protozoan infections, we attempted to examine more closely the mechanism of this resistance to reinfection in our cured animals.

TABLE III

Animals		Date of infection	Date of treatment	Dose per kilo	Reinfections: Days after treatment 1	2	3	4	Remarks
Rabbit	7	10.6.23	2.7.23	0.1	29	65	82	132*	20 days after last reinfection parasites appeared in the blood.
,,	8	25.6.23	2.7.23	0.1	29	65	—	—	22.10; 110 days after treatment animal died, when blood was drawn from heart. Infection negative.
,,	14	26.11.23	16.12.23	0.05	35	99	160*	—	After third reinfection, infection developed and followed a normal course.
Guinea-pig	1	8.4.23	27.4.23	0.2	44	76	95	131, 178	10.11; 197 days after treatment animal well.
,,	8	21.6.23	2.7.23	0.2	29	65	—	—	18.9; 78 days after treatment animal died of pneumonia.
,,	9	25.6.23	15.7.23	0.15	16	52	—	—	5.10; 82 days after treatment animal died while being bled.
,,	35	10.12.23	27.1.24	0.05	56	116	—	—	Died from extraneous cause 146 days after treatment; no infection.
,,	38	10.12.23	27.1.24	0.05	56	116	—	—	Observed 170 days, still alive and well.
,,	104	17.1.24	17.2.24	0.10	36	120	—	—	No infection, died after observation 147 days, from sunstroke.
,,	106	28.1.24	18.4.24	0.06	39	—	—	—	Alive and well, no signs of infection.

* Parasites appeared only after an injection of oil.

Humoral antibodies.—It is well known that the serum of rats which have recovered spontaneously from the infection with *T. lewisi* has the power to agglomerate trypanosomes. A similar condition has not been observed in animals infected with pathogenic trypanosomes. Levaditi and McIntosh (1910) have reported the

presence of a trypanocidal antibody in trypanosome infected animals following a pyrexial period. It appeared, therefore, of interest first of all to note whether there were any of the humoral antibodies in our cured and resistant animals. For this purpose a series of experiments were performed of which the following are examples. Each experiment was repeated at least two or three times, but only type experiments are given below :—

Experiment 1. Sera were collected from treated animals at intervals of 3 to 8 weeks after recovery. These sera were diluted with saline in various proportions up to 1 : 10 and suspensions of live trypanosomes added. The suspensions of trypanosomes were prepared as follows :—5 c.c. of blood from a heavily infected animal were taken from the heart and mixed with an equal volume of 1 per cent. citrated Ringer solution. The red cells were then sedimented by slow centrifugation—about 600 revolutions for 5 to 6 minutes. The supernatant fluid contained a uniform suspension of trypanosomes. Definite quantities of the suspensions were added to the sera and the tubes placed at 20° to 25° C. Observations for motility and agglutination were made with the dark field microscope, at various intervals, from 1 to 48 hours. Controls were made with sera from normal animals and from infected animals taken shortly after pyrexial crisis.

In none of the experiments was there any indication of injury to, or even retardation of, the motility during the first six hours. In all of the higher concentrations of serum up to 1.5, actively motile trypanosomes were seen even after an incubation of 48 hours. In general the sera from cured resistant animals reacted in the same manner as did normal sera.

Experiment 2. This experiment was an attempt to apply the Pfeifer reaction to the trypanosomes. Sera from resistant animals were mixed with suspensions of trypanosomes made in the manner described above in the ratio of 5 to 10 parts of serum, respectively, to one part of suspension. The mixtures were injected intraperitoneally into guinea-pigs and, at intervals, material was withdrawn for dark field observation.

There were two variations to this experiment.

Experiment 2a. Two guinea-pigs were each inoculated with 1·0 c.c. serum from cured guinea-pig No. 1 and at the same time 0·1 c.c. of infected blood was injected. A control animal was treated in the same manner with normal serum. Material was withdrawn from the peritoneal cavity at intervals of half, 1, 2, and 18 hours for dark field examination. The trypanosomes remained actively motile, although after 18 hours the stained preparations of the trypanosomes from all the animals showed vacuolations. After an incubation period of 10 to 11 days trypanosomes appeared in the circulation.

Experiment 2b. Sera from three cured resistant animals were pooled and 5 c.c. quantities mixed in varying ratios with trypanosome suspensions. The suspensions were incubated for one hour at 25° C. and then injected into guinea-pigs of approximately the same weight. At intervals similar to those in Experiment 2a, specimens were withdrawn with a capillary pipette and examined under the dark field microscope.

There was no apparent effect on the motility during the twenty-four hours after the injection. Seven to eight days after the injection trypanosomes appeared in the circulation of all of the animals used for the experiment.

This series of experiments indicated that the blood of resistant animals did not contain any trypanocidal antibody which could be demonstrated either *in vitro* or *in vivo*.

Cellular factors in resistance to reinfection.—Immunity is usually considered a two-fold phenomenon—cellular and humoral. In the absence of any demonstrable humoral antibodies we directed our attention to the cellular changes occurring during the infection and subsequent to treatment. It was noted that marked and striking cellular changes occur. The following table (Table IV) gives a summary of white cell counts of a series of animals before and during the infections and before and after treatment. It will be noted that during the infection there is a moderate but uniform leucopaenia with a preponderance of lymphocytes, and that immediately after treatment there is a sharp increase in the total leucocyte count and in the large mononuclears, and a tendency to a reversal of the ratio to normal. This increase is usually greater than normal and the condition persists in all the cured animals for a considerable period.

THE BEARING OF RELAPSES TO RESISTANCE TO REINFECTION.—It was noted above that the course of the disease in the mule as well as in the experimental animals is intermittent in character ; pyrexia accompanied by the appearance of trypanosomes in the circulation is followed by apyrexial periods of greater or lesser duration. In the rabbit these apyrexial intervals are much greater than in the guinea-pig and occasionally a rabbit may develop a severe type of chronic infection with emaciation, loss of hair, conjunctivitis, keratitis, etc., and yet parasites may be rarely encountered in the peripheral circulation.

The explanation of the relapsing nature of the infection is obscure, but it is undoubtedly bound up in some way with the resistance of the animal host to the invading virus. It appears likely that if not the same, at least similar factors play a rôle in the two phenomena : the resistance to blood invasion and resistance to reinfection ; and that an elucidation of the factors determining the appearance of relapses might throw light on the nature of the immunity.

TABLE IV

Blood picture in Experimental Trypanosomiasis before and after Treatment*

No. of animal		Average Counts							
		During incubation				During infection			
		Total	Polymorphs	Lymphocytes	Mononuclears	Total	Polymorphs	Lymphocytes	Mononuclears
Guinea-pig	104	11,300	65	34	4	8,200	46	52	3
,,	107	8,200	60	35	3	7,000	40	58	2
,,	112	11,100	68	31	3	7,000	52	42	5
Guinea-pig	32	8,000	52	45	3	8,000	37	58	5
,,	33	10,000	60	35	2	10,000	48	49	2
,,	35	9,400	58	40	2	9,200	41	56	3
,,	38	8,400	63	35	2	...	...	...	...
Rabbit	8	8,000	55	43	2	6,500	29	68	3
,,	11	8,000	60	38	2	7,500	54	44	2
,,	18	8,000	58	40	2	7,000	38	60	2
,,	14	9,000	65	33	1	7,400	37	61	2

No. of animal		Average Counts							
		After treatment				Immediately after 'Bayer 205'			
		Total	Polymorphs	Lymphocytes	Mononuclears	Total	Polymorphs	Lymphocytes	Mononuclears
Guinea-pig	104	10,000	58	38	4	10,000	78	16	6
,,	107	7,800	60	39	4	8,000	68	24	7
,,	112	9,700	61	36	3	9,200	45	40	15
Guinea-pig	32	12,000	51	45	4	10,900	38	53	9
,,	33	10,200	63	32	5	8,600	37	54	9
,,	35	...	...	...	...	9,200	38	57	5
,,	38	9,800	53	43	4	12,200	48	47	5
Rabbit	8	11,000	56	42	2	11,000	56	40	3
,,	11	11,500	51	46	3	9,200	58	35	7
,,	18	9,700	47	51	2	10,200	60	34	6
,,	14	10,000	45	52	3	9,900	60	36	4

* The averages are obtained from approximately ten counts made during the infection or after the treatment, and the entire incubation period. It should be noted that during the pyrexial periods there is a marked lymphocytosis which gradually changes to a normal differential during the apyrexial period. In treated animals the maximum total counts are obtained two or three days after the injection of the drug. During the first few days the lymphocytes and large mononuclears predominate, but gradually the cell distribution approaches normal, although the total count may be considerably above the normal average.

Assuming that this interplay of host resistance to virus is associated with the cellular mechanism, we attempted to induce relapses artificially by agents which are known to affect the white cell equilibrium. The work of Bergel (1921) and the results obtained by Nakahara (1922) in his studies on cancer, indicated that oil, particularly olive oil, may produce marked changes in the white cell formula and in the resistance of the host to an invasion of foreign bodies. We consequently used a variety of oils with striking results.

ARTIFICIAL PRODUCTION OF RELAPSES.—Relapses were produced at will by the injection of oils, such as cod liver or olive oil, particularly the latter. Ordinary commercial olive oil was employed. The oil was sterilized in the autoclave and injected intraperitoneally. As the protocols indicate, the effect of the oil has been tried on a fairly large series of animals under various conditions and the results have been unfailing in their constancy.

The following are brief extracts of the protocols :

*A. Experiments with guinea-pigs.**

1. *Effect of small doses of olive oil :*

GUINEA-PIG 13. Infected 1 July ; infection of long duration ; parasites intermittently present in the circulation. On 11 November parasites negative ; 0·25 c.c. sterile olive oil given intraperitoneally. 12 November large number of trypanosomes in the blood ; persistent until 16 November, then negative until 30 November ; 0·25 c.c. oil again given. On 3 December trypanosomes positive and increased steadily in number assuming appearance of continuous infection, until 16 December when treated.

GUINEA-PIG 26. Infected 22 October ; 26 October blood positive ; remittent infection. On 11 November rare parasites present in the circulation ; given 0·25 c.c. of olive oil i.p. ; on 12 November trypanosomes positive and number increased until 14 November, when the trypanosomes suddenly disappeared, and the blood continued negative.

GUINEA-PIG 28. Infected 22 October ; parasites infrequently present in small numbers. On 11 November blood negative ; 0·25 c.c. olive oil given intraperitoneally ; on 12 November trypanosomes appeared in the circulation, persisted until 15 November when treated.

2. *Effect of large doses of oil.*

GUINEA-PIG 12. Infected 19 July ; trypanosomes appeared in the blood on 29 July ; present at irregular intervals for varying lengths of time ; infection unusually long duration. On 11 November trypanosomes were negative ; 4 c.c. olive oil injected i.p. ; 12 November trypanosomes found in the blood. Infection

* The weights ranged from 450 to 500 gms.

positive to 15 November, then negative until 25 November and intermittent. On 30 November another injection of 4 c.c. oil given, 2 December after two days, there was a large increase in the number of parasites and they persisted to the end on 18 December.

Guinea-pig 24. Infected 22 October; 26 October blood positive; intermittent infection. 11 November few parasites in the circulation; injected 4 c.c. olive oil; 12 November large increase in numbers; trypanosomes persisted for seven days and then disappeared. After the blood was negative 11 days, another injection of 4 c.c. given; two days later parasites reappeared in the blood and persisted until 16 December, when treated.

Guinea-pig 27. Infected 22 October, remittent infection. On 11 November blood negative; 4·0 c.c. olive oil injected intraperitoneally. 12 November trypanosomes present in the circulation; persisted until 15 November when animal treated.

Guinea-pig 31. Infected 10 December, trypanosomes in blood 16 December; then negative until 15 January. That day 4 c.c. olive oil injected intraperitoneally; 16 January trypanosomes appeared in the blood and continued present until 17 February when treated.

Guinea-pig 36. Infected 10 December; 18 December blood positive. Examined at intervals until 15 January and no parasites found. On 15 January 4 c.c. olive oil given i.p. On 16 January trypanosomes appeared in the circulation and continued positive until 3 February, when enormous numbers were found in the blood and animal treated.

Guinea-pig 38. Infected 10 December. Blood continually negative. On 15 January 4 c.c. olive oil given i.p.; next day trypanosomes appeared and persisted with two days intermission until 27 January when treated.

B. Experiments with Rabbits.

Rabbit Y. Weight 1390 gms. Infected 5 September. Blood positive 17 September. Parasites rare in circulation; present only five times in two months. On 11 November 5 c.c. olive oil given i.p. On 13 November parasites appeared in circulation, increased in numbers until 18 November; then negative for five days; then increasingly positive until 27 November when the animal died with a heavy blood infection—a most unusual occurrence in rabbits.

Rabbit 4. Weight 1650 gms. Infected 5 November, course of infection irregular; relapses at irregular intervals. On 21 December 10 c.c. olive oil given i.p.; 22 December, trypanosomes present; infection continuous until the animal died.

Rabbit 19. Weight 1250 gms. Infected 10 November, acute conjunctivitis and loss of hair, but trypanosomes constantly negative. On 25 February, 4 c.c. olive oil given i.p.; on 27 February blood was positive and continued so until 3 March, when treated.

The preceding protocols indicate that olive oil injected intraperitoneally into infected guinea-pigs and rabbits was followed usually within twenty-four to forty-eight hours, by an appearance of trypanosomes in the peripheral circulation. The immediate results are not affected by the amount of oil injected; but there seems to be

some relationship between the amount of oil and the persistence, or continued multiplication, of the trypanosomes in the blood. The smaller doses (0·25 c.c.) were followed by a relapse, the persistence of parasites for three or four days, followed by their disappearance for a greater or lesser interval. The larger doses of oil were also followed by the appearance of trypanosomes in the circulation within twenty-four to forty-eight hours after the injection, but the persistence was apparently longer than was the case with the small doses, and in many of the animals the resistance seemed to have been broken down completely. Whenever parasites were present in the body the injection of olive oil served to cause a flare-up and an invasion of the circulation. Rabbits reacted in the same way as guinea-pigs.

Suppression of Resistance in Cured Animals.—Proceeding on the hypothesis that there is a relationship between the resistance to blood invasion and that to reinfection, we attempted to determine whether it is possible to break down the resistance in the same manner as it was possible to produce relapses. Into animals cured from an infection were injected varying doses of olive oil in a manner similar to that used to produce relapses. The results were not uniformly successful, but in a few cases we actually succeeded in breaking down the resistance and calling out an infection which had apparently remained latent. The observations are given in the following protocols :

Rabbit 11. Weight 1750 gms. Injected 26.11.23 ; on 29.11.3, 22 c.c. of bile injected intraperitoneally. Trypanosomes appeared 1.12.23, the rabbit was treated with 0·05 gm. ' Bayer 205 ' per kilo. The blood was rendered negative, and the blood picture changed. On 15 January 4 c.c. oil was given intraperitoneally. On 16 January trypanosomes were found.

Note. The drug had not destroyed the trypanosomes, but rendered the infection latent. A dose of oil caused a flare up of the infection.

Rabbit 7. After three superinfections with negative results (see Table III) this rabbit was given (on 11.11.23) 6 c.c. oil intraperitoneally together with an infective dose of trypanosomes. No trypanosomes appeared in the circulation. On 30.11.23 a similar dose of oil was given intraperitoneally, and on 1.12.23 trypanosomes were found in the blood. From that day trypanosomes appeared intermittently until 23.12.23, when the animal died from the infection.

Note. In this case it appeared that the oil broke down the resistance and called out the infection which was latent for three weeks.

Rabbit 14. Weight 1690. Infected 26.11.23, treated (0·05 gm. per kilo) 16.12.23. Re-infected 20.1.24, results negative. On 25.3.24, 5 c.c. oil together with 0·1 c.c. infected blood given intraperitoneally. No parasites found in the blood. On 14.4.24 another dose of oil given and on 17.4.24 trypanosomes appeared

in the circulation. They then disappeared until 28.5.24, and from then on continued to appear intermittently until the end.

These results could not be duplicated in guinea-pigs treated with ' Bayer 205.' The reason is not apparent, but it would seem that oil is only effective in mobilizing the trypanosomes when the infection is present in a latent state in the body. In any event, the positive results obtained in rabbits increase the presumption that the resistance to reinfection is a temporary phenomenon bound up in some way with the cellular elements and differing from the type of immunity observed in bacterial infection.

IV. DISCUSSION

An outbreak of trypanosome infection in mules in Northern Palestine afforded an opportunity for an experimental study of trypanosomiasis in lower animals, particularly rabbits and guinea-pigs.

The morphology of the parasite, the clinical picture of the disease, and the pathological lesions produced, indicate that the strain belonged to the *T. evansi* group.

In the course of these studies the therapeutic effect of ' Bayer 205 ' and the mode of action of this drug were investigated. The drug proved very effective in clearing up the infection in dogs, rabbits and guinea-pigs ; a single dose of 0·05 gm. per kilo of body weight was sufficient to sterilize the blood in all instances and apparently affected permanent cure in 80 per cent. of the rabbits and guinea-pigs so treated.

The mechanism of the action of the drug is of interest. *In vitro* it has very little trypanocidal power ; even a concentration of 1 : 100 requires twenty-four to forty-eight hours to immobilize the parasites under the condition of the test. *In vivo*, on the other hand, so small a dose as 0·05 gm. per kilo suffices to sterilize the blood of a heavily-infected animal in about sixteen hours. It would seem that the drug is either modified in the animal body and rendered more effectively trypanocidal, or that it acts indirectly on the trypanosomes by increasing the resistance of the host. The fact that a small amount of the drug injected into normal animals affords protection against infection for considerable periods, is further presumptive

evidence that the drug either enters into combination with the tissues and is retained in the body for a long time, or that it produces profound changes in the body leading to increased resistance to infection. Which of these alternatives occur it is difficult to say ; it is most likely that both events actually take place.

Experimental evidence is advanced in favour of the view that a real immunity or resistance to infection is set up in the body of animals cured from an infection. Even in normal healthy animals there is a partial resistance to an infection with this strain, as indicated by the relapsing nature of the infection. In the course of the infection, the resistance offered to the invasion of the blood stream is repeatedly broken down (probably by substances liberated by the parasites which continue their activity in the tissues), and the trypanosomes penetrate into the circulation and go through a period of active development. The destruction of the trypanosomes in the circulation leads to a partial immunization resulting in an inhibition of growth and a disappearance of the parasites from the circulation.

This immunity or resistance seems to be associated with the formed elements of the blood. Repeated search failed to reveal any of the usual humoral antibodies (agglutinins, lysins). On the other hand, profound changes occur in the white blood cell formula during the infection, and after the treatment. Moreover, it is possible to break down the resistance artificially by the injection of olive oil, a substance shown by various authors to modify the white cell ratio. This observation, namely, the calling forth of relapses by the injection of olive oil, is analogous to the breaking down of the resistance of mice to transplanted cancer by the injection of large doses of olive oil reported by Nakahara (1923). Since, in the latter instance, the resistance has been shown to be due to a proliferation of cells in the lymphoid tissues, and the suppression to a decrease in the lymphoid cells, there is reason to assume that the same or similar factors are involved in the resistance to trypanosome invasions. In this connection, our observation that the injection of ' Bayer 205 ' causes a marked increase in the white cell count, particularly the large mononuclears, is significant. The defence of the body against trypanosome infections, and perhaps also to other protozoan infections such as malaria, seems, therefore, to lie in the cellular response rather than in the humoral antibodies.

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NOTES ON SOME TETRARHYNCHID PARASITES FROM CEYLON MARINE FISHES

BY

T. SOUTHWELL

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The material on which this paper is based was collected by the author from various species of Elasmobranch fishes trawled on the Ceylon Pearl Banks during the years 1906 to 1911. A large number of larval forms were also obtained from bony fishes, but, owing to the fact that the proboscides were only rarely extruded, it has not been possible to identify them except in one or two cases. Unfortunately the major part of the writer's collection has been lost and no other material is obtainable; several species obtained by Herdman and Hornell from the same region have been described by Shipley and Hornell in Herdman's Ceylon Pearl Oyster Reports. As far as the writer is aware the only Trypanorhynchids available are in the collections of Professor Pintner of Vienna and Professor Linton in America.

A very large number of Tetrarhynchids have been described from time to time, often from larval forms; the descriptions are, however, so imperfect that it is impossible to identify the majority of these worms again from the meagre details given. Very few of the type species at present exist, with the result that there is great confusion. Until large quantities of material have been collected it will not be possible to revise this Order satisfactorily. The writer has merely attempted to place the classification of the TRYPANORHYNCHA on as satisfactory a basis as is possible in the present state of our knowledge and, in addition, has given a description, for the first time, of the anatomy of a few species.

The writer is indebted to Professor James Johnstone, Oceanographical Department, University of Liverpool, for specimens of two or three species.

CLASSIFICATION

Rudolphi (1809) erected the genus *Tetrarhynchus* and included it in his Order **Cestoidea ;** he ascribed to the genus the following characters :—

'Body flat and continuous; head furnished with two bipartite bothridia with four retractile armed proboscides.'

In the year 1819 Rudolphi erected the genus *Rhynchobothrius* to include *Bothriocephalus corrolatus* and *B. paleaceus*, but he did not define its characters.

Van Beneden (1850) included the genus *Tetrarhynchus* in his *TETRAPHILLES* and in his division PHYLLORHYNCHIENS; he defined the characters of the family as follows :—

'These animals are characterised in the first place by the four bothridia which are common to the whole section and to which correspond four retractile proboscides, armed with hooks disposed in spirals and lodged in a membranous sheath. There is always a distinct neck; the longitudinal canals traverse the entire length of the head and strobila, commencing in the interior of the bothridia.'

He referred to the genus thus :—

'As I only know one genus in this family it is useless to enumerate its characters.'

Van Beneden further stated that the genus *Tetrarhynchus* included those forms which, in the larval stage, are destitute of a vesicle, and that the genus *Rhynchobothrium* included mature worms possessing segments. As the name *Tetrarhynchus* has priority he considered the name *Rhynchobothrius* to be a *nomen nudum*.

Diesing (1850) placed the *RHYNCHOBOTHRIA* as a sub-tribe of the *BOTHRIOCEPHALIDEA* and divided it into the following genera :—

Dibothriorhynchus with two opposite bothridia ; body continuous.

Tetrarhynchus with two opposite bothridia, each bothridium being divided by a longitudinal septum.

Rhynchobothrium with two opposite bothridia ; body articulated.

Tetrabothriorhynchus with four bothridia, opposite, in pairs, apices converging.

Stenobothrium ; with four lateral opposite bothridia, body continuous.

Tetrarhynchobothrium ; with four lateral opposite bothridia ; body segmented.

Synbothrium ; with four terminal, cruciform, opposite bothridia joined to the head posteriorly by a membrane.

The same author in 1863 classified the Tetrarhynchids as follows :—

Section PARAMECOCOTYLEA.

Tribe *Paramecocotylea aprocta.*

Sub-tribe *Trypanorhyncha.* Head with four boring proboscides armed, and retractile into the neck.

Family DIBOTHRIORHYNCHA (characters not defined).

'Genus *Rhynchobothrium.* Body articulated ; head with two bothridia facing each other, lateral or marginal, complete and divided by a longitudinal septum ; four armed proboscides. Neck tubular ; male pore marginal, female lateral, or both marginal. In intestine of marine fishes.'

Family TETRABOTHRIORHYNCHA.

'Body articulated ; head with four lateral bothridia facing each other in pairs, or standing out terminally, disposed crosswise ; four free armed proboscides ; or singly traversing the bothridia ; neck tubular. Genital pores marginal or lateral. Parasitic in marine fishes.

Genus *Tetrarhynchobothrium.* Body articulated ; head with four lateral bothridia opposite each other in pairs ; four free proboscides. Pores marginal or lateral. Parasitic in marine fishes.

Genus *Syndesmobothrium.* Body articulated ; head with four terminal prominent bothridia placed crosswise ; four proboscides, each one running through the middle of a bothridium. Pores marginal (?). Parasitic in marine fishes.'

Carus (1863) included the genus *Tetrarhynchus,* Cuvier (?) in the sub-family 'PHYLLORHYNCHIDEA,' van Ben., of the Family 'TETRAPHYLLIDEA.' He stated that the characters of the genus were similar to those of the sub-family, viz. :—

'The scolex is separated from the strobila by a neck, and has four suckers, sometimes joined in pairs ; four proboscides always armed with hooks which can usually be drawn back into sheaths.'

In 1864, Cobbold erected the family TETRARHYNCHIDAE with characters as follows :—

'The members of this family are easily recognised by the possession of four armed retractile proboscides attached to the head. The armature consists of several successive rows of sharply-pointed, recurved hooks, frequently amounting to several thousands. The head itself is usually more or less bilobed, each half supporting either one bipartite bothrium, or else two separate fossae. These cavities are also frequently supported on four petaloid appendages, which vary much in shape in the different species, and also in the same individual, according to the degree of contraction of the part. The head and neck are continuous, and usually about the same breadth as the body, the latter being sometimes even narrower than either the head or neck. The body is depressed, filiform, distinctly segmented, and usually of great length in the mature state, the reproductive orifices being situated at the lateral margin of the joints in an irregularly alternate manner.'

Linton, 1889, sub-divided the family as shown below :—

Family TETRARHYNCHIDAE, Cobbold, 1864.

Synonyms :—Sub-tribe *Trypanorhyncha*, Diesing, 1863.
Sub-family *Phyllorhynchinae*, van Ben.

Sub-family I. DIBOTHRIORHYNCHINAE, Mont., 1892.

Synonym :—*Dibothriorhynchidae*, Dies.

Genus *Rhynchobothrium*, Rudolphi, 1819.

Synonym :—*Tetrarhynchus* of authors.

'Body taeniaeform. Neck tubular. Head continuous with neck, with two opposite bothria, parallel or converging at the apices, lateral or marginal, entire or undivided, or either bilocular with a longitudinal partition, or bilobed or divided. Proboscides four, terminal, filiform, armed, retractile in the neck, for the most part longer than the head. Genital apertures, male marginal, female lateral, or male and female marginal approximate.'

Genus *Otobothrium*, Linton, 1889.

'Body articulate, taeniaeform, head separated from body by a neck. Bothria two, opposite, lateral, each with two supplemental ciliated pits at the posterior free angles. Proboscides four, terminal, filiform, armed, retractile in neck. Reproductive apertures marginal.'

Sub-family II. TETRABOTHRIORHYNCHINAE, Mont., 1888.

Synonym :—*Tetrabothriorhynchidae*, Dies., 1863.

Genus *Tetrarhynchus*, Rudolphi, 1809.

Synonyms :—*Bothriocephali* spec., Bartels ?
Rhynchobothrii spec., van Ben. and R. Leuckart.
Tetrarhynchi spec., van Ben.
Aspidorhynchus, Molin, 1858.
Tetrarhynchobothrium, Dies.

'Body articulate, taeniaeform. Neck tubular. Head with four bothria in two lateral pairs, parallel with the head. Proboscides four, terminal, filiform, armed, retractile in the neck, free, *i.e.*, not running through the bothria. Genital apertures marginal or lateral.'

Genus *Syndesmobothrium*, Diesing, 1854.

'This genus is characterised by Diesing as follows :—Body articulate taeniaeform ; neck tubular, rounded at the base ; head tetragonal, with four terminal prominent bothria attached to head by posterior margin, cruciformly disposed, oval, slightly convex, joined with each other at the base by a membrane ; proboscides four, filiform, armed, each one running through a bothrium (pedicel) excurrent at apex, long, retractile in the neck. Genital apertures marginal (?). In the intestines of marine fishes of tropical America.'

Loennberg, in 1889, erected the COENOMORPHINAE as a sub-family of the TETRARHYNCHIDAE. Type and only species *C. grossus* (Rud.) = *T. linguatulus* (van Ben.) = *T. solidus*, Drummond, 1838. Larvae have been recorded in Decapods.

The principal characters of the sub-family are :—(1) the presence of a double set of genitalia in each segment, and (2) the fact that the worms are very stout and muscular.

Braun (1900) defined the Order **Trypanorhyncha,** Diesing, 1863, as follows :—

'The scolex is divided into head and neck ; head with two or four bothridia and with four retractile and armed proboscides ; segmentation complete, segments usually dividing off before maturity. Pores marginal or sub-marginal, uterine pore ? Genitalia as in TETRAPHYLLIDEA. Larvae in different marine animals, adults usually in the guts of Plagiostomes.'

He placed the following genera in the family; an abstract of his definitions is given below :—

Rhynchobothrius, Rud.

This is the oldest genus, erected to accommodate the species *Bothriocephalus corollatus* and *B. paleaceus*, Rud. The author only saw drawings of these species. He discusses the question as to whether *B. corollatus* and *Taenia corollata*, Fab., are synonymous, but says too little is known to decide. *R. corollatus* is selected as the type species. It has two simple bothridia with four proboscides at the four corners, each proboscis being furnished with 20 to 30 hooks bent posteriorly.

Dibothriorhynchus, de Blainv., 1824 (as appendix to French translation of Bremser).

The drawing shows an unsegmented cestode with a posterior tubercle, two bothridia with a posterior longitudinal septum, two short, retractile proboscides armed with small, bent hooks. Later on the worm was named *D. lepidopteri*. The author considers two proboscides a mistake of the observer, or else, according to Loennberg, it was a larval form. Loennberg's observations enable the following amended description to be given :—

'TRYPANORHYNCHA with four, short, thick, club-shaped or half-circular retractile proboscides armed with hooks ; and two sessile, powerful suckers ; segmentation complete ; segments very muscular, always broader than long, not dividing off ; genitalia double in each proglottis, each set having three pores ; cirrus and vagina marginal, uterus ventral. Larvae not encysted ; adult in stomach of Selachiens.'

Diesing set up another genus *Dibothriorhynchus* for *Tetrarhynchus scolecinus*, Rud., and *T. gracilis*, Rud. (*i.e.*, larval forms), but adult forms have also received the name ; they really belong to the genus *Rhynchobothrius*. Thus Diesing's *Dibothriorhynchus* is a synonym of *Rhynchobothrius*.

Tetrarhynchobothrium, Dies. Type species *T. tenuicolle*, Diesing, but four other species were added to this genus later on.

Characters of the genus are :—Cylindrical neck, four bothridia, four thread-like proboscides, and irregularly alternating marginal pores ; ripe segments longer than broad. *Tetrabothriorhynchus*, Dies., is a synonym of this genus.

Aspidorhynchus, Mol.

Contains one species only, viz., *A. infulatus*, Mol. Diesing placed it in the genus *Tetrarhynchobothrium*. Molin's characteristics include the form and position of the bothridia and a telescopic, retractile neck ; these characters are not sufficient to justify the retention of genus *Aspidorhynchus*.

Synbothrium, Diesing. Diesing later on changed the name to *Syndesmobothrium*.

Characters :—Four oval, convex bothridia placed crosswise on the surface of the head and bound together by a basal membrane ; four, thread-like proboscides, which traverse the longitudinal axes of the bothridia and emerge anteriorly. Genital pores marginal. *Synbothrium fragile*, Dies., was for a long time the only species, but Linton described *S. filicolle*, though he has not described it sufficiently, as the specimen was not mature. Host :—*Trygon centrura* (spiral valve).

Abothros, Welch. Only species :—*A. carcharias*, Welch. from *Carcharias*, sp.

Chief characters :—Alleged absence of bothridia ; the four, slender proboscides are armed with hooks bent backwards, emerging close together on the ventral surface of the head. Segments very short. Genital pores ? Certain longitudinal furrows could be seen on the scolex, probably the missing bothridia. *A. carcharias*, Welch, is probably identical with *Bothriocephalus bicolor*, v. Nordm.

Otobothrium, Linton. The only species is *O. crenacolle*, Linton.

Characters :—Two ventral bothridia with longitudinal septum, each of which bears on its free posterior edge, two little suckers. Four, thread-like proboscides, pores marginal.

Lühe (1910) defined the Order **Trypanorhyncha** thus :—

'Cestodes whose scolex is usually continued into a *Kopfstiel* ; with two or four bothridia at whose apical end are four armed extensile proboscides. When retracted (with the assistance of a retractor which runs in their interior and is inserted into their anterior end) each is drawn back into a proboscis sac. This corresponds in thickness and length with the proboscis itself and represents a direct continuation of the proboscis into the anterior end of the scolex and *Kopfstiel*. At its inner end, the sheath passes directly into the visibly thicker, sharply delineated, egg-shaped or sausage-shaped sac, whose contraction brings about the extrusion of the proboscides. Outer segmentation complete. Formation of segments as in TETRAPHYLLIDEA ; mature in stomach or spiral valve of Selachiens ; larvae found in all kinds of marine animals. In fresh water only a few species are found in the larval condition as parasites of Teleosts. No details of the deveplopment of the larvae are known.'

Lühe distinguished two families, *viz.* :—

1. Larva encysted ; proboscis long, slender, cylindrical, whole body not massive or muscular..... *Tetrarhynchidae*, Lühe, 1910
2. Free larvae, not encysted ; probocsis short ; almost semi-globular or club-shaped ; whole body robust and muscular..... *Coenomorphidae*, Lühe, 1910

He ascribed the following characters to the TETRARHYNCHIDAE :—

'Scolex with long, slender, cylindrical, very mobile proboscides, with two or four very mobile bothridia more or less leaf-like. *Kopfstiel* present. Strobila slender, with little muscular development; often transparent. Segments, when mature, longer than broad, easily detachable; in each segment a single set of genital organs. Uterus apparently without primary pore. Ripe eggs, as in TETRAPHYLLIDEA, escape through dehiscence. In spiral valve of Selachiens; larvae in Turtles, Bony fish, Cephalopods and Decapods.'

He added that nothing was known regarding the systematic division of the family.

The family COENOMORPHIDAE, he defined as follows:—

'Scolex very robust with short, thick proboscides, semi-globular or club-shaped, with two simple bothridia sunk into the scolex like a pit or a split, and with edges which hardly protrude; no *Kopfstiel*. Strobila robust and very muscular, up to 4 mm. in thickness and not transparent; segments when mature much broader than long and not separating off. In each segment there are two sets of genital organs; uterus with a special pore opening ventrally and having its own muscular system. Mature in stomach of sharks; larvae, not encysted, found in bony fish. There is only one genus with one species, viz., *C. grossus* (Rud.) = *T. solidus* = *T. linguatulus*.'

Pintner (1913) in dealing with Tetrarhynchids in general, pointed out that our information relating to the anatomy of the various forms was not sufficient to enable one to deal extensively with the order, as only six species appear to be well-determined, *T. ruficollis*, Eysenh., 1829, being the best known. Specific points are hardly ever mentioned by authors who have hitherto described different species. The principal characters in distinguishing species, according to Pintner, are :—

(1) The scolex; but this is not altogether definite; the length of the part of the bothridium attached to the head is of importance, the peduncle behind the head is included as scolex. (2) The exact number and shape of hooks. (3) The form of the proboscis sacs. (4) Whether the head is separated from the neck. (5) The general appearance of the worm. (6) The specific characteristics of ripe segments. (7) The presence or absence of a uterine pore.

Pintner identified three groups, viz. :—

1. With a true uterine pore present ..... *T. viridis* group
2. With an involuted, apparent pore, not found in anterior segments ..... *T. ruficollis* group
3. Segments dehiscent, no uterine pore either primary or secondary ..... *R. tenuis* group

He defined a number of genera as follows :—

Eutetrarhynchus, Pintner, 1913.

' Scolex very long and slender, with small proboscides. The two flat, spoon-like bothridia are deeply embedded (in lateral view) and inclined to the long axis at about 45°. Proboscis bulb at least twice as long as the rest of the scolex ; in this respect it resembles *T. longicollis*. The proboscides are very long and thick and are covered with a pile of small uniform hooks which look black in low magnifications owing to their small size, but this is not so when highly magnified. Proboscis not longer than proboscis sheath. The retractor lies in the fundus of the *Kolben* (knob) and is formed of parallel, closely arranged fibres in which the large constituent cells, for their whole length, and in large numbers, are arranged on one side. The very long knobs are formed of only five or six broad shells of clearly striped, transverse muscle bands, in a single layer, with large spherical myoblasts arranged on the inner side of the knobs in the form of stripes. Neck short ; chain weak but markedly craspidot, seldom apolytic. Generally one or two very mature segments, or longer pieces of greater age, become detached from the chain. Frequently they retain the primary end segment. Segments at the end of the chain sometimes longer than broad. Genital pore in the middle of the margin ; apparent uterine openings in the middle line on a level with the pore. The oviduct opens far in front into the uterine sac. Testes large and very numerous, occupying all the segment. Type species :—*Eutetrarhynchus ruficollis* (Eysenhardt, 1829). *E. leucomelanus*, Shipley and Hornell, 1906, also belongs to the same genus.'

Stenobothrium, (Diesing, 1863).

' Four bothridia (two dorsal and two ventral) whose sides are furnished with very long (but not projecting) hairs ; proboscides very long. In *S. macrobothrium* they are the longest of any known *Tetrarhynchid*, being several times as long as the rest of the cephalic region. Scolex markedly craspidot ; proboscides weak, short, thin and thread-like, with uniform, not numerous, small hooks, rather far apart. The sheaths are correspondingly thin and short and they bear small knobs. The knobs are formed of very numerous thin muscles in six shells, each of which consists of many muscle layers, the innermost being the thinnest. In transverse section the knob muscles appear not to be arranged as usual, but to lie parallel like four horses racing side by side, the thinnest part of each muscle representing the head and the thickest part the hind end of the horse ; retractor one-celled. A single, large, constructive cell occurs between the fibrillae, and these have no nuclei ; their surface is covered with minute (chitin-like ?) papillae. Frontal glands powerfully built. Strobila strongly developed. Even the last segments broader than long, with completely smooth sides, laterally limited by a straight line. Genital cloaca situated at the anterior end of the segment towards the ventral side ; the long cirrus sac stretches the length of the anterior margin of the segment, its end being bent backwards. Uterine openings in the middle line behind one another. Vitelline glands not so dense as the testes generally are, because the ventral sides are occupied by the coils of the uterus. Ovary of two dorsal and two ventral wings, which, on each side, are sub-divided into five or six lobes ; the tubes connecting these sub-divisions are very thin and the wall consists of a single layer of cells ; a muscular septum is present at the end of each segment. At the primary hind end (especially in the larva) there projects a conical knob from inside the bladder which is thickly covered with strong hairs, (viz., *S. macrobothrium*) or with papillae (viz., *S. linguale*). Type species :—*S. linguale*, Cuvier, 1817. Other species :—*S. macrobothrium* (Rudolphi, 1819), *T. bisulcatum*, Linton, 1889, *T. tenue*, Linton, 1890, *T. robustum*, Linton, 1890, *S. herdmani*, Shipley and Hornell, 1906, *S. perideraeum*, Shipley and Hornell, 1906. One of Linton's species is probably identical with *S. linguale* Cuvier, 1817.'

Lakistorhynchus, Pintner, 1913.

'Head small and fragile; delicate, fine, and very pointed angle-shaped hooks occur on the soft thin proboscides. Strobila euapolytic or hyperapolytic; the segments grow a lot after separation. They are very long and, in the chain, pass very suddenly from unripe to ripe. The genital atrium is situated in the middle of the segment and is distinguished by a sucker-like pit in front and behind it. The surface of the ripe segments bears marked papillae. The proboscis sheath affords characters which are peculiar in two ways. Whereas in *benedeni* they are long and form spirals (in connection with a very contractile pars vaginalis) so that the proboscides, even in a completely withdrawn condition, do not approach by a long way the muscle knobs; in *platycephalus* and *rubromaculatus*, on the contrary, the sheaths are short and, therefore, also not in spiral, and the proboscides when fully drawn in, actually extend from the part bearing the hooks to the middle of the hollow space of the muscle knobs; consequently, the retractor in them is arranged in very dainty stripes. This divergence in scolex structure can easily be used as of generic importance. At times, also, the species are very peculiar. On the other hand there is between *benedeni* and *platycephalus* such a similarity in proglottides, and in the case of *benedeni* and *rubromaculatus* such a correspondence in the scolex that I must, for the present, place them in the same genus. Type species:—*L. benedeni*, Crety, 1890. Other species:—*L. rubromaculatus*, Diesing, 1863, *L. platycephalus*, Shipley and Hornell, 1906.'

Halsiorhynchus, Pintner, 1913.

'Probably one has to make a new genus for Shipley and Hornell's *T. ruficollis*, type species *H. shipleyanus* as the chief characters are the proboscis hooks and the "coat of mail." It is like a species I described as *Rhynchobothrius vario-uncinatus*. Shipley and Hornell's form has not the slightest resemblance to *T. ruficollis* (Eysenhardt, 1829). The chief characteristic of Shipley and Hornell's species is the armoured chain of proboscides hooks.'

Sphyriocephalus, Pintner, 1913.

'Species thick and muscular; two large, sucker-like bothridia, one dorsal and one ventral, giving the head a hammer-like appearance. Extruded proboscides rigid, thick and straight; proboscis sacs transverse; anterior extremity pointing outwards. The *attenuatus*-group shows the same character, which is also common in the larva, but in the larvae the posterior end of the sacs turns outward. Scolices craspidot; hooks almost equal in size and in 20 transverse and 16 longitudinal rows. The little hooks measure 0·009 mm. and 0·02 mm., the hooks are slightly larger in the middle, viz., 0·045 to 0·075 mm.; bases 0·048 mm. Eggs in one species are bell-shaped with two enormous processes at the poles, one is long, the other is small (47 to 65μ long and 6μ broad at base) and claw-shaped. Egg 75μ × 47μ, one side being flattened. Type species:—*S. viridis*, Wagener, 1854. Another species:—*S. turgistensis*, Pintner, 1913.'

Attenuatus group.

Pintner did not name or define the group, but he included in it the following:—

'Genus *Coenomorphus*, Loenn., 1889; also *T. attenuatus*, Rudolphi, 1819, *T. grossus* (Rudolphi, 1819) and *T. megalocephalus*, Shipley and Hornell, 1906.

'*T. equidentatus*, Shipley and Hornell, 1906, also possibly belongs to the *attenuatus* group, or it is a transitional form between the *attenuatus* group and *Stenobothrium*.'

Otobothrium, Linton, 1890.

'Type species *O. crenecolle*, Linton, 1890. *T. carcharidis*, Shipley and Hornell, 1906, is possibly synonymous with the type species.'

Pintner gave the following synonymy for the species mentioned below :—

Tetrarhynchus leucomelanus, Shipley and Hornell, 1906 = *Eutetrarhynchus leucomelanus.*
,, *herdmani*, Shipley and Hornell, 1906 = *Stenobothrium herdmani.*
,, *tenue*, Linton, 1890
,, *bisulcatum*, Linton, 1889 } = All *Stenobothrium* sp.
,, *robustum*, 1890
,, *platycephalus*, Shipley and Hornell, 1906 = *Latistorhynchus platycephalus.*
,, *benedeni* = *T. tenius* = *T. gracilis* = *L. benedeni.*
,, *ruficollis*, Shipley and Hornell, 1906 = *Tetrarhynchus shipleyanus* = *Halysiorhynchus shipleyanus* = *Rhynchobothrius vario-uncinatus.*

The same author adopted the terminology noted below :— The head is *craspidot* where there is a division between the head and the neck. It is *acraspidot* where the division is absent. Ripe segments are *anapolytic*, when they remain attached to the strobila. Ripe segments are *apolytic* when they automatically separate from the strobila. Gravid segments are *euapolytic* when they separate from the chain and continue to grow. Gravid segments are *hyperapolytic* if they separate from the strobila before they are mature and especially if they do so before the uterus is developed.

The table given below summarises the details relating to the bothridia in the different genera according to the authors noted below.

Until more material is available for examination the writer proposes the following classification.

Order **Trypanorhyncha,** Diesing, 1863, emended.

Family I. COENOMORPHIDAE, Lühe, 1910, emended.
Genus *Coenomorphus*, Loennberg, 1889, emended.

Family II. TETRARHYNCHIDAE, Cobbold, 1864, emended.
Genera :—*Tetrarhynchus*, Rud., 1809, emended.
Rhynchobothrius, Rud., 1819, emended.
Syndesmobothrium, Diesing, 1863.
Otobothrium, Linton, 1890.

The characters of the order, families and genera are given below.

TABLE I

Table of Genera

Author	Genus	Bothridia
Rudolphi, 1809 ...	*Tetrarhynchus*	Two bothridia; bipartite
Rudolphi, 1819 ...	*Rhynchobothrius*	Two simple bothridia
Blainville, 1824 ...	*Dibothriorhynchus*	Two simple bothridia
Van Beneden, 1849	*Tetrarhynchus*	Four bothridia
Diesing, 1850 ...	*Dibothriorhynchus*	Two opposite bothridia; body continuous
Diesing, 1850 ...	*Tetrarhynchus*	Two opposite bothridia, each bothridium being divided by a longitudinal septum
Diesing, 1850 ...	*Rhynchobothrium*	Two opposite bothridia; body articulated
Diesing, 1850 ...	*Tetrabothriorhynchus*	Four bothridia, opposite; in pairs apices converging
Diesing, 1850 ...	*Stenobothrium*	Four lateral opposite bothridia; body continuous
Diesing, 1850 ...	*Tetrarhynchobothrium*	Four lateral opposite bothridia; body segmented
Diesing, 1850 ...	*Synbothrium*	Four terminal cruciform opposite bothridia joined to head posteriorly by a membrane.
Diesing, 1863 ...	*Rhynchobothrium*	Two bothridia divided
Diesing, 1863 ...	*Tetrarhynchobothrium*	Four bothridia
Diesing, 1863 ...	*Syndesmobothrium* (*Synbothrium* renamed)	Four terminal bothridia
Linton, 1889 ...	*Rhynchobothrium* (= *Tetrarhynchus* of authors)	Two bothridia; entire or sub-divided
Linton, 1889 ...	*Otobothrium*	Two bothridia with posterior ciliated pits
Linton, 1889 ...	*Tetrarhynchus*	Four bothridia
Linton, 1889 ...	*Syndesmobothrium*	Four terminal bothridia
Braun, 1900 ...	*Rhynchobothrius*	Two bothridia
Braun, 1900 ...	*Dibothriorhynchus*	Two bothridia each with a septum
Braun, 1900 ...	*Tetrarhynchobothrium*	Four bothridia
Braun, 1900 ...	*Synbothrium* = *Syndesmobothrium* ...	—

It will be seen from the above table that the genus *Tetrarhynchus*, as originally described by Rudolphi, had two bipartite bothridia and that he applied the name *Rhynchobothrius* to forms with two simple bothridia.

Van Beneden was the first to state definitely that the genus *Tetrarhynchus* possessed four bothridia, although the fact is implied in Rudolphi's definition of the genus.

The synonymy of the two genera is therefore as follows :—

Tetrarhynchus, Rudolphi, 1809.

Synonyms :—*Tetrarhynchus*, van Ben., 1849.
Tetrarhynchus, Diesing, 1850.
Tetrabothriorhynchus, Diesing, 1850.
Stenobothrium, Diesing, 1850.
Tetrarhynchobothrium, Diesing, 1850 and 1863.
Rhynchobothrium, Linton, 1889, pro parte.
Tetrarhynchus, Linton, 1889.
Dibothriorhynchus, Braun, 1900.
Tetrarhynchobothrium, Braun, 1900.

Rhynchobothrius, Rudolphi, 1819.

Synonyms :—*Dibothriorhynchus*, Blainville, 1824.
Dibothriorhynchus, Diesing, 1850.
Rhynchobothrium, Diesing, 1850.
Rhynchobothrium, Linton, 1889, pro parte.
Rhynchobothrius, Braun, 1900.

Although it is easy to refer worms with two or four bothridia to their respective genera, there are a few species in which each bothridium is only *partially* divided and it may then become difficult to decide whether there are two or four bothridia. Such forms are to be regarded as intermediate and may be referred to either genus.

Characters of the Order **Trypanorhyncha,** Diesing, 1863, emended.

Head with two or four bothridia, and bearing four retractile proboscides armed with hooks ; segmentation complete. Genital organs as in the *TETRAPHYLLIDEA*. Adult in marine Elasmobranch fishes and occasionally in Teleosts. Larvae in Teleosts and invertebrates. With two families.

Family I. TETRARHYNCHIDAE, Cobbold, 1864, emended.

Trypanorhyncha with a single set of genitalia in each segment. Worms more or less fragile. Parasitic in marine and fresh water fishes.

Family II. COENOMORPHIDAE, Lühe, 1910, emended.

Trypanorhyncha possessing a double set of genitalia in each segment ; strobila stout and muscular. Parasitic in marine and fresh water fishes.

KEY TO GENERA

A single set of genitalia in each segment.....1
A double set of genitalia in each segment..... *Coenomorphus*

1. Head with four bothridia.....2
Head with two bothridia.....3

2. Bothridia parallel with body and with posterior half free..... *Tetrarhynchus*
   Bothridia terminal, cruciform, at right-angles to body; attached to head by posterior margin..... *Syndesmobothrium*
3. Bothridia with ciliated pits at their posterior margin..... *Otobothrium*
   Bothridia without ciliated pits; each bothridium often more or less divided into two..... *Rhynchobothrius*

Genus *Tetrarhynchus*, Rudolphi, 1809, emended.

Head with four bothridia arranged in pairs, and lying parallel with the head.

Tetrarhynchus tetrabothrius (van Ben., 1849).

Several specimens from *Acanthia vulgaris*, North Sea, August, 1923, collected by the author; also from the dog-fish, Isle of Man, collected by Mr. Birtwistle.

Tetrarhynchus perideraeus, Shipley and Hornell, 1906 (figs. 1 to 3).

This species, according to Shipley and Hornell, measures up to 70 mm. in length and 1·3 mm. in breadth. The head bears two lappets, but they are so divided in the centre as to appear like four; the proboscides are slender and bear oblique rows of very minute teeth all of uniform size; the proboscis tubes and proboscis sheath

FIG. 1. *Tetrarhynchus perideraeus*. A hook. × 1125.

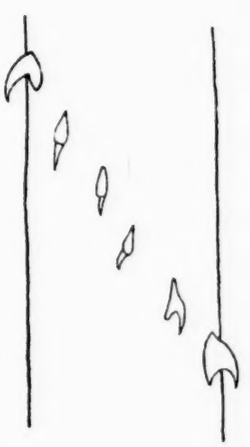


FIG. 2. *Tetrarhynchus perideraeus*. A row of hooks on proboscis. × 500.

are also short. The head is produced backwards into a very characteristic collar which overlaps and embraces the anterior part of the body. The neck is fairly long; the segments have straight sides, except posteriorly; the middle portion of the strobila shows a

tendency to become coiled or twisted ; the genital pores are irregularly alternate and are situated about the middle of the lateral margin. The species was obtained from the intestine of *Carcharias gangeticus*.

The writer obtained a few specimens of this species from the intestine of *Ginglymostoma concolor*, Pearl Banks, Ceylon, 27th February, 1909, and adds the following notes on the anatomy of the species :—

External Anatomy. The *head* measures about 1·36 mm. in length and 0·85 mm. in breadth ; the hooks are spirally arranged and measure 16μ ; neck absent. There are a large number of segments having convex margins ; the genital pores are lateral and irregularly alternate, being situated a little in front of the centre.

Internal Anatomy. *Testes.* The testes vary in number from 60 to 70 ; they occupy the entire dorsal area within the

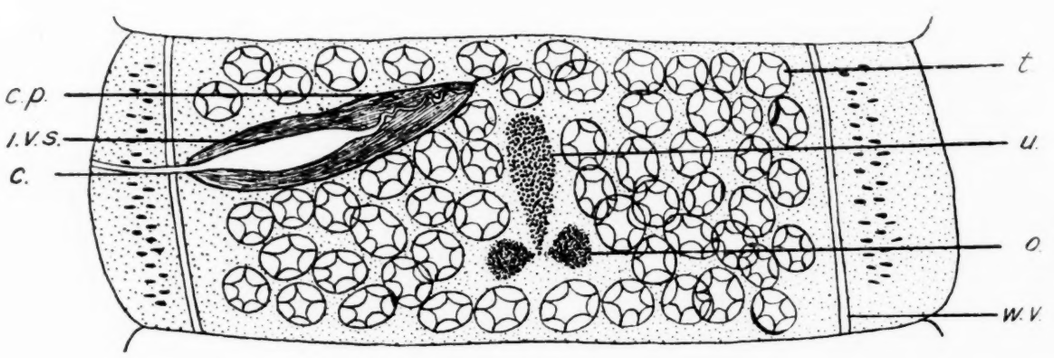


Fig. 3. *Tetrarhynchus perideraeus.* Mature segment. *t.*—testes ; *u.*—uterus ; *o.*—ovary ; *w.v.*—water vessel ; *c.*—cirrus ; *i.v.s.*—internal vesicula seminalis ; *c.p.*—cirrus pouch. × 112.

excretory vessels and a few testes are situated posterior to the ovary.

Vas deferens. The cirrus pouch is conspicuous and lies anterior to the vagina and median to the excretory vessels ; it communicates with the exterior by means of a long narrow duct. In the median direction it extends almost half-way across the segment, its median extremity being closely apposed to the anterior extremity of the segment ; no spines were observed on the cirrus. The vas deferens lies coiled within the cirrus pouch, near the median extremity of which it dilates into a seminal vesicle.

Ovary. This is peculiar in being situated some distance from the posterior extremity of the segment, and in being small and dumb-bell

shaped ; it stains very deeply and the two lobes are very compact. The vagina runs posterior to the cirrus pouch.

Vitelline glands. These are very scanty and consist of single acini practically encircling the segment.

Uterus. This consists of a sac with irregular walls which completely fills the segment ; it was full of immature eggs.

Tetrarhynchus aetobatidis, Shipley and Hornell, 1906.

One specimen, immature, was collected by the author from the intestine of *Trygon kuhli*, Ceylon Pearl Banks, 1909.

This species is easily identified on account of the fact that (1) the head is swollen, the swelling being due to the stout muscular proboscis sacs ; (2) there is a deposit of pigment (present in preserved specimens) at the junction of the proboscis tubes with the proboscis sacs.

As the specimen was immature, the anatomy is not known.

Tetrarhynchus sp.

The larval form of the species about to be discussed occurs in the Ceylon Pearl Oyster and was originally believed to be concerned in pearl production. The writer has recently (1924) summarised our knowledge relating to the pearl-inducing parasite in the Ceylon pearl oyster, except with regard to the *Tetrarhynchus* larva. At least three different species of larval cestodes inhabit the tissues of the pearl oyster. Two of these are globular and belong to the genus *Tylocephalum*, whilst the third is elongated and belongs to the genus *Tetrarhynchus*. There can be no doubt that the larval parasites concerned in pearl production belong to the former genus ; the larval Tetrarhynchid is confined almost exclusively to the gut wall of the oyster ; Shipley and Hornell (1904) described the adult of this larva as occurring in *Rhinoptera javanica*, and they named the adult worm *Tetrarhynchus unionifactor*. All the three larval forms found in the pearl oyster were believed by Shipley and Hornell, without sufficient reason, to be young forms of the adult *Tetrarhynchus unionifactor* found in *Rhinoptera javanica*. For reasons given by the writer in the paper referred to above, the specific name *unionifactor* is now restricted to the adult form of the larger globular larva found

in the pearl oyster, which is now known as *Tylocephalum unionifactor ;* in all probability *Tylocephalum dierama*, Shipley and Hornell, 1906, is a synonym of *T. unionifactor.* The Tetrarhynchid larva found in the pearl oyster is therefore without a name.

As a result of feeding sharks and rays with infected pearl-oysters the writer, on two occasions, obtained large numbers of an adult Tetrarhynchid, which, at the time, he believed to be identical with *Tetrarhynchus unionifactor*, Shipley and Hornell, 1904.

Unfortunately, nearly all the material has been lost and it is therefore impossible to describe the adult worm until more material is available. The adult worms obtained by the writer differ, however, from the worm described by Shipley and Hornell in having four instead of two bothridia, and in the hooks not being all alike as they are stated to be in Shipley and Hornell's worm.

Professor Th. Pintner, of Vienna, has been good enough to examine the fragments of adult worms obtained by the writer from *Ginglymostoma concolor*, as a result of feeding experiments, and he is of opinion that the species belongs to his genus *Latistorhynchus* because (1) the head is small and delicate, (2) the proboscides are long, the hooks fine and pointed, and (3) the proboscis sheaths are spiral. The scarcity of material did not allow of a definite identification, but the species is close to, but different from *L. benedeni*, Crety, 1890. Shipley and Hornell state that in their larval form of *Tetrarhynchus unionifactor*, the body is covered with warts and this feature is, according to Pintner, one of the characteristics of the genus *Latistorhynchus.* It is quite possible that Shipley and Hornell's species, and that obtained by the writer, belong to the same genus, but they are certainly different species, and the writer's specimens are closely related to, if not identical with, *Tetrarhynchus rubromaculatus* (Diesing), Shipley and Hornell, 1906.

Genus *Rhynchobothrius*, Rud., 1819, emended.

Body taeniaeform. Head with two lateral or dorso-ventral bothridia, each bothridium being either entire, or partly divided.

Rhynchobothrius erinaceus (van Ben., 1858).

SYNONYMS :—*Rhynchobothrium imparispine*, Linton, 1890.
Rhynchobothrium simile, Linton, 1909.
Tetrarhynchus gangeticus, Shipley and Hornell, 1906.
Tetrarhynchus annandalei, Hornell, 1912.

Very numerous specimens from the spiral valve of *Trygon* sp., Ceylon Pearl Banks, 1910. Collected by the author.

Shipley and Hornell, in 1906, under the name *T. macroporus*, described a worm which appears to differ from *R. erinaceus* only in having the bothridia

'Each divided into two, each half corresponding with one of the four hooked proboscides.'

The writer has had the opportunity ot examining the type (and only) specimen of *R. annandalei* obtained from *Stegostoma tigrinum*.

The proboscides were not extruded and the shape of all the hooks could not be determined ; it was noted, however, that they were of various shapes, many of them being large. The testes are numerous and the pores are irregularly alternate, large, and situated in the posterior third of the segment. The worm is indistinguishable fror. *R. erinaceus*.

Rhynchobothrius macrocephalus (Shipley and Hornell, 1906) (fig. 4).

SYNONYMS :—*Tetrarhynchus macrocephalus*, Shipley and Hornell, 1906.
Tetrarhynchus ruficollis, Shipley and Hornell, 1906.

Numerous specimens from the intestine of *Rhynchobatus djeddensis*, Ceylon Pearl Banks, February 3rd, 1911 ; also from *Trygon walga*, November 27th, 1910. Collected by the author.

Shipley and Hornell described this species from a young form which was quite immature, and which measured 8 mm. only in length. The head measured 6 mm. in length and the rest of the body 2 mm. They called attention to the fact that the distinctive character of this species was

'The herring-bone spicules on the proboscides and the grading of the hooks on the same.'

Pintner, who examined the type species, states that *T. macrocephalus* is the same as the mature worm measuring 40 mm. to 50 mm., identified by Shipley and Hornell, as *T. ruficollis* (Eysenhardt, 1829), and with this statement the present writer is in agreement. Pintner

further points out that the worm identified by Shipley and Hornell as *T. ruficollis* (Eysenh.) is quite a different species from that described by Eysenhardt.

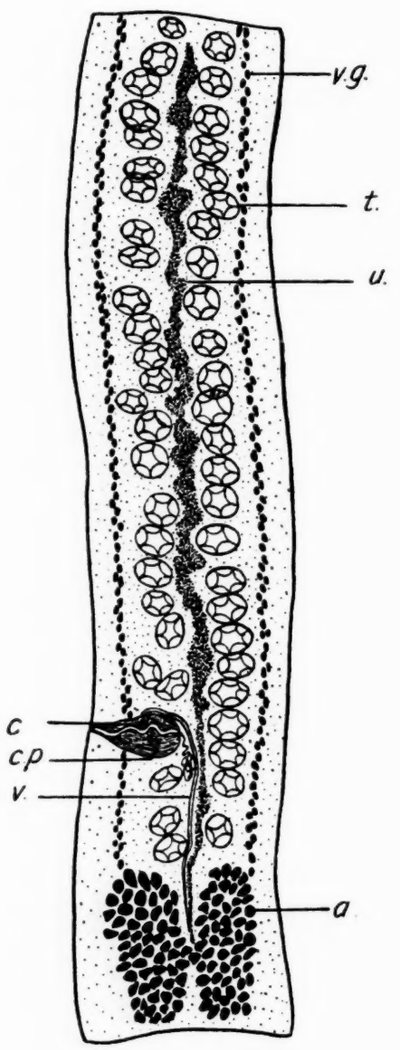


FIG. 4. *Rhynchobothrius macrocephalus.* Mature segment. *v.g.*—vitelline glands; *t.*—testes; *u.*—uterus; *c.p.*—cirrus pouch; *c.*—cirrus; *o.*—ovary; *v.*—vagina. × 69.

Pintner erects a new genus which he names *Halsiorhynchus* to accommodate Shipley and Hornell's *T. macrocephalus* and he also changes the specific name to *shipleyanus*.

For reasons which are stated elsewhere, the writer is of opinion that Pintner's classification cannot be accepted until more is known regarding the morphology of the **Trypanorhyncha** generally, and

further, the changing of the specific name to *shipleyanus* is clearly contrary to the accepted rules of nomenclature. Pintner states that

> 'One has to make a new genus for Shipley and Hornell's *T. ruficollis* (type species *Halsiorhynchus shipleyanus*) as the chief characters are the proboscis hooks some of which are arranged like " a coat of mail or armoured chain " (herring-bone type). It is like a species I described as *Rhynchobothrius vario-uncinatus*. Shipley and Hornell's form has not the slightest resemblance to *T. ruficollis* (Eysenh.).'

As *T. macrocephalus* was described first, and as it is identical with the worm which Shipley and Hornell called *T. ruficollis* (Eysenh.), it is clear that the specific name *macrocephalus* must stand.

As the anatomy of the worm has not been described, the following notes are now added :—

The worms measure up to 50 mm. in length ; there is nothing to add to the description of the head except that there are two bothridia only. There is no neck. The worm is composed of about thirty-five segments and the genital pores are irregularly alternate and situated in the posterior third of the segment. There are about fifty-five testes ; the *cirrus pouch* extends almost to the median longitudinal axis of the segment. The *ovary* is bilobed, each lobe consisting of about fifteen large acini. The *oviduct* is short and appears to run anterior to the pouch. In segments mounted entire, the *vitelline glands* appear to consist of a single row of acini running along the lateral margins, but they actually encircle the entire segment. The *uterus* at first consists of an irregular tube running in the median antero-posterior axis of the segment. It eventually becomes bag-shaped and entirely fills the segment.

Rhynchobothrius rhynchobatidis, Shipley and Hornell, 1906 (figs. 5 to 9).

Synonyms :—*Tetrarhynchus rhynchobatidis*, Shipley and Hornell, 1906.
Rhynchobothrium curtum, Linton, 1909.

Three specimens from the intestine of *Trygon sephen.*, Ceylon Pearl Banks ; collected and presented by James Hornell, Esq., F.L.S.

External Anatomy. The worms are relatively large and stout, measuring up to 50 mm. in length and having a maximum breadth of 1·5 mm. They are composed of numerous thick segments, with slightly salient posterior margins, the last segment measuring 2 mm. in length and 1·4 mm. breadth ; the genital pores are irregularly alternate and are situated in the posterior third of the segment.

Head. The head is very small and somewhat heart-shaped, the pointed extremity being directed anteriorly. It has a maximum width of 750μ and a length of 850μ. The two bothridia are small, having a breadth of 530μ and a length of 470μ; their margins are entire and only slightly thickened. The proboscis sacs are situated almost immediately behind the posterior margins of the bothridia and they have a length of 245μ and a maximum breadth of 110μ. The neck measures about 220μ in length; anteriorly it is somewhat thickened and the posterior extremity of the proboscis sacs lie in the thickened portion. The armed portion of the proboscides and the proboscis sheaths are each about as long as the bothridia.

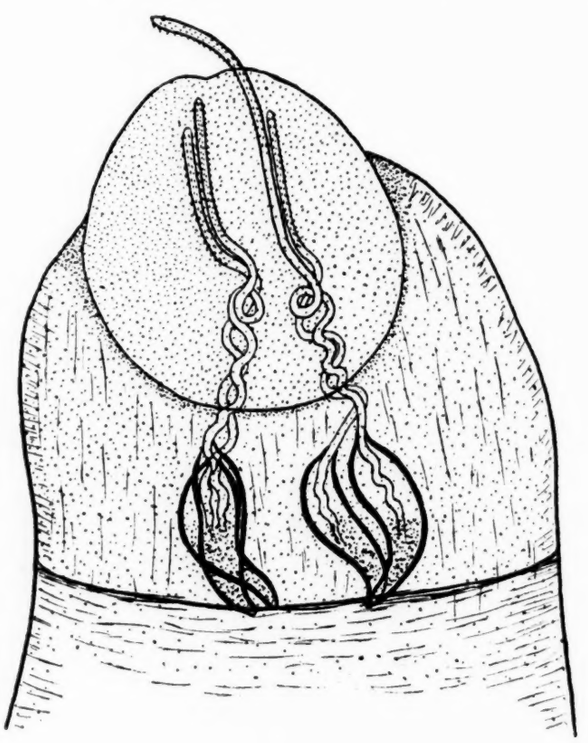
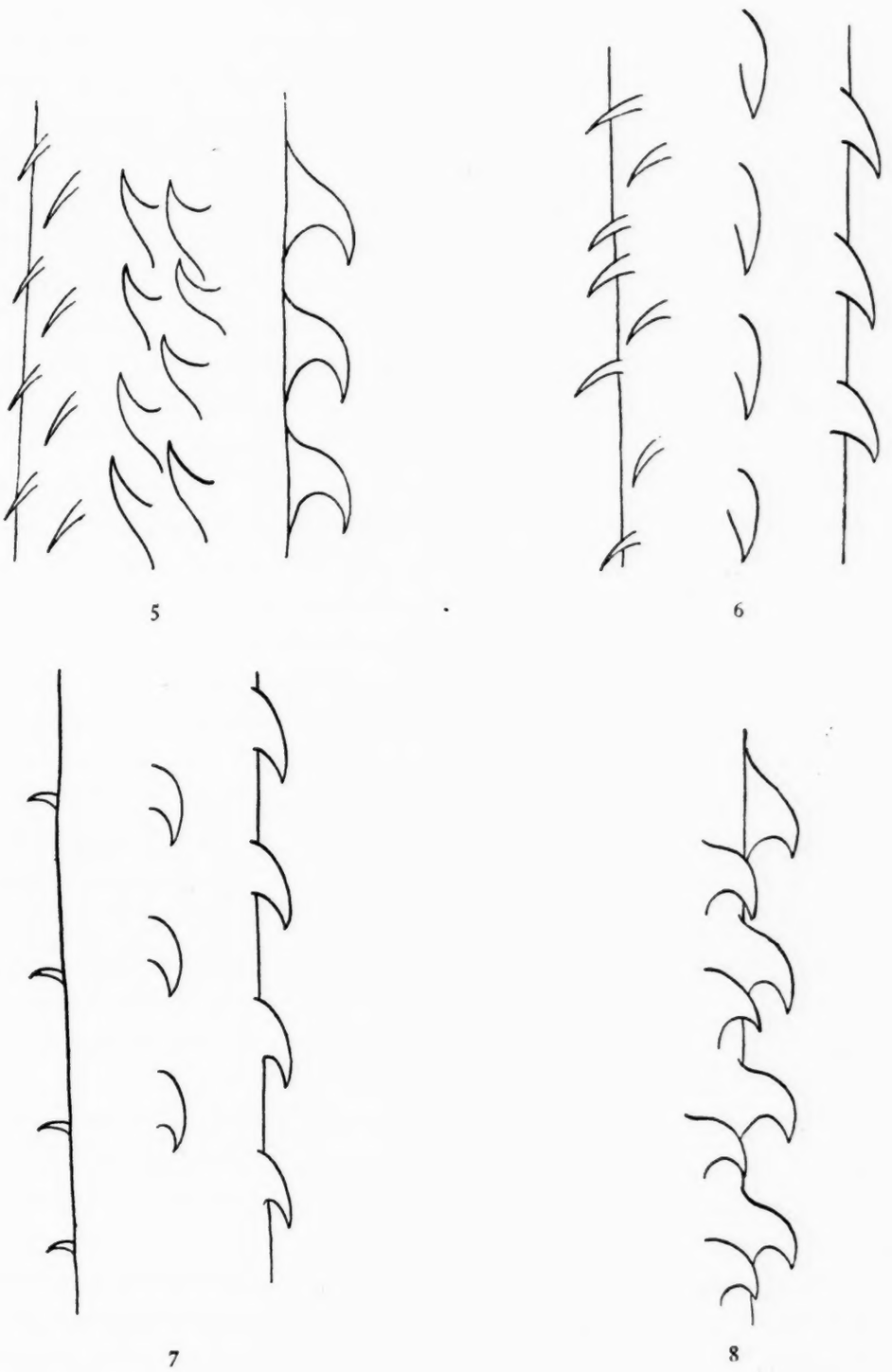


FIG. 9. *Rhynchobothrius rhynchobatidis.* Head. × 69.

Hooks. The hooks on the proboscides appeared to be arranged irregularly and not, as is usual, in rings or in spirals. The internal face of each proboscis bears a longitudinal row of rose-thorn shaped hooks 8μ in length arising from a base also 8μ in length. Ventrally there are three or four longitudinal rows of smaller rose-thorn shaped hooks, two longitudinal rows of which have their points directed anteriorly instead of posteriorly; in Shipley and Hornell's specimens,



FIGS. 5 to 8. *Rhynchobothrius rhynchobatidis.* Hooks from various parts of proboscis. × 1125.

only one longitudinal row of hooks pointed anteriorly. Dorsally there are two more rows of small rose-thorn shaped hooks gradually changing in shape both dorsally and ventrally into about two longitudinal rows of very slender, sabre-like hooks, also 8μ in length on the external face of each proboscis. The neck is very short, measuring only 200μ in length.

INTERNAL ANATOMY. *Muscular system.* The longitudinal muscular system is strongly developed and consists dorsally and ventrally of a single row of oval bundles. Laterally the bundles are much smaller and scattered about irregularly.

Excretory system. This consists of two vessels on each side, the internal vessel is large and the external vessel (which lies directly external and close to the large vessel) is very small.

Details of the nervous system were not investigated. The cortical and medullary parenchyma is strongly developed.

Genitalia. Testes. These are very numerous and fill the dorsal part of the segment in front of the ovary. In the early stages of development they are crowded together in the median field, on each side of the mid-antero-posterior axis. The cirrus pouch is conspicuous and extends one-third the distance across the segment ; no spines were observed on the cirrus. Posterior and median to the pouch the vas deferens forms a number of conspicuous coils.

Ovary. This is, as usual, a bilobed organ situated posteriorly ; the vagina is a short, coiled tube which runs ventral (?) to the pouch and opens to a shallow genital atrium.

Shell gland. This is a conspicuous organ lying posterior to the ovary.

Vitelline glands. These completely encircle the segment and are situated in the cortical parenchyma.

Uterus. This arises as a closely coiled tube running to the extreme anterior margin of the segment ; eventually it entirely fills the segment, and is distended with eggs, none of which, however, were mature.

DIAGNOSIS. This species resembles *T. rhynchobatidis* generally, and in particular in having certain hooks on the proboscides pointing anteriorly, and in the pore being situated posteriorly. It appears to differ from it, however, in the shape of the head generally, in the form of the bothridia, and in having a double row of hooks pointing

anteriorly. The worm has also a somewhat close resemblance to that of *R. curtum*, Linton, 1909, from which it only differs : (1) in being much larger, (2) in the relative size of the bothridia to the rest of the head, and (3) in the form and size of the posterior segments.

The armature of the proboscides in *R. curtum* has not been described.

These differences, however, do not appear to the author to be of much specific value and accordingly the worm described above, and also Linton's *R. curtum*, are considered identical with *T. rhynchobatidis*, Shipley and Hornell, 1906.

Rhynchobothrius longicollis (van Ben., 1849) (fig. 10).

SYNONYM :—*Tetrarhynchus leucomelanus*, Shipley and Hornell, 1906.

One specimen of what appears to be this species was obtained by the author from *Trygon sephen*, Portugal Bay, November 7th, 1910 ; other specimens from *Rhynchobatus djeddensis*, February 3rd, 1911 ; *Trygon kuhli* and *Trygon walga*, November 27th, December 3rd, 1910, and February 7th and February 21st, 1911.

Shipley and Hornell gave the following diagnosis of this species :—

'Five centims. to eight centims. long, with posteriorly thick, stout proglottides, three millims. broad. Anterior half or two-thirds of the preserved body white, the remainder slaty black, deepening into a dense black. When alive, milky white, with a pink patch behind the proboscis sheath. Head with shallow lappets, well defined. Proboscides with an enormous number of very minute teeth, all uniform size and shape, arranged in rings and longitudinal rows. The proboscis sacs are very long, occupying seven-tenths of the length of the head. There is a short neck ; the posterior edge of each proglottis is salient. Generative pores irregularly alternate. Habitat :—Intestine of *Trygon sephen*.'

The specimens agree closely with Shipley and Hornell's description of this species. The hooks on the proboscides are all alike and are extremely minute. As the anatomy of this species has not been described, the following notes are added :—The genital pores are irregularly alternate and are situated a little distance behind the middle of the lateral margin of the segment.

Testes. The testes vary in number from about 165 to 210 ; they occupy the entire dorsal area in front of the ovary. Aporally there are from about 90 to 110 testes ; and porally there are from about 25 to 40 testes posterior to the cirrus pouch, and about 50 to 60 anterior to that organ.

Vas deferens. The cirrus pouch is small and almost globular, not measuring more than one-fifth the transverse axis of the segment. From the preparations it was impossible to decide whether the cirrus was armed or not. Median to the pouch the vas deferens forms a conspicuous coiled mass.

Ovary. This is a large bilobed organ situated posteriorly. Details relating to the vagina could not be made out in whole mounts.

Vitelline glands. These encircle the entire segment except for a small area dorsally and ventrally.

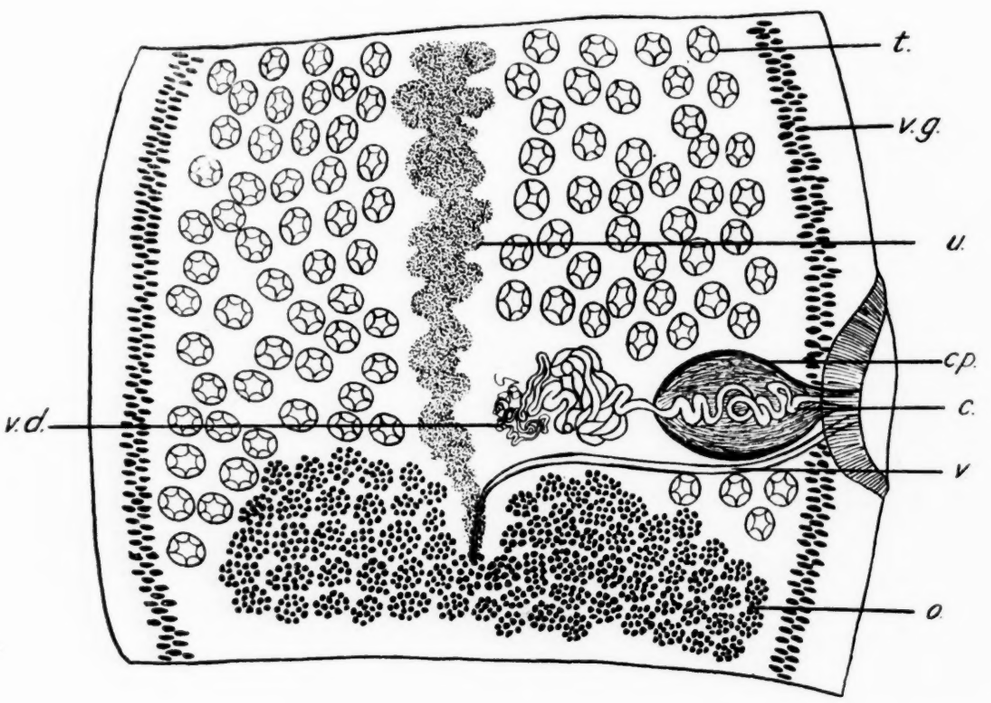


FIG. 10. *Rhynchobothrius longicollis.* Mature segment. *t.*—testes; *v.g.*—vitelline glands; *u.*—uterus; *c.p.*—cirrus pouch; *c.*—cirrus; *v.*—vagina; *o.*—ovary; *v.d.*—vas deferens. × 69.

Uterus. At first this consists of a coiled tube running in the median line from the ovary to the extreme anterior margin of the segment. When fully developed it consists of a bag with sacculated walls entirely filling the segment. The uterus was full of immature eggs.

Shipley and Hornell's species is indistinguishable from, and *T. ruficollis* (Eysenh., 1829) is closely related to, *Tetrarhynchus longicollis*, van Ben., 1849.

Rhynchobothrius spinuliferus (Southwell, 1911).

SYNONYMS :—*Tetrarhynchus spinulifera*, Southwell, 1911.
Rhynchobothrium laciniatum, Yoshida, 1917.

Two specimens were examined, taken from the intestine of *Rhynchobatus djeddensis*, Ceylon Pearl Banks, 1908.

EXTERNAL ANATOMY. The worm measures up to 5·5 cms. in length and the greatest breadth is 1 mm. It is composed of a large number of segments, the last measuring about 1·3 mm. in length. The posterior margins of the segments are produced into long digitate flaps with pointed extremities ; these laciniae are small in the neck region and short and blunt in gravid segments. The pores are situated laterally at the junction of the anterior two-thirds and posterior third of the segment. A uterine pore was present on the ventral surface ; the segments do not leave the chain until the uterus is fully mature. The neck is short, measuring only about 250μ.

Head. The head is very small, measuring 1 mm. in length ; its breadth at the bulbs is 120μ, and in the vicinity of the sheaths, 76μ ; the two bothridia measure 126μ in length and 90μ in breadth ; the proboscis sheaths form long, dense, spiral coils, and the proboscis sacs measure 280μ in length and 27μ in breadth. Unfortunately, the proboscides were not protruded and consequently details relating to the spines cannot be given. The entire head is covered with very minute spinules.

INTERNAL ANATOMY. Owing to the scarcity of material the muscular, excretory and nervous systems were not examined.

Testes. The number of testes could not be counted, as the worms were not well-preserved.

Vas deferens. The cirrus pouch is large, extending to the median longitudinal axis of the segment. The cirrus is dilated near the pore, and a number of coils of the vas deferens lie within the pouch. Outside the pouch the vas deferens forms a small coiled mass near the median extremity of the pouch.

Ovary. This is a bilobed organ situated posteriorly and comprised of a few, large, club-shaped acini. When fully mature the acini appear to fuse on each side, giving the ovary a dumb-bell appearance.

Vagina. Unfortunately, details relating to this organ could not be made out.

Vitelline glands. These encircle the entire segment and are composed of large acini.

Uterus. This develops early as a tube with very thick lobulated lateral walls ; it eventually fills the entire segment. A uterine pore is situated ventrally near the median extremity of the cirrus pouch ; it has a muscular margin.

Eggs. No fully ripe eggs were seen ; of those observed some were flask-shaped, with a number of short filaments at one end, whilst others were somewhat kidney-shaped, with a number of short filaments at both extremities.

There is no room for doubt that *R. laciniatum,* Yoshida, 1917, is identical with *R. spinuliferus* (Southwell, 1911).

Rhynchobothrius carcharidis, Shipley and Hornell, 1906.

Synonym :—*Tetrarhynchus carcharidis,* Shipley and Hornell, 1906.

Two specimens of what appears to be this species were obtained from the intestine of *Trygon walga,* Ceylon Pearl Banks, 1910 ; collected by the author.

External Anatomy. The worms measured up to 25 mm. in length and the greatest breadth was about 1 mm. ; the last segment, which was gravid, measured 1·2 mm. in length, and 1 mm. in breadth ; in all, about ninety segments were counted under magnification, but only the last ten or so were relatively large. The genital pores are irregularly alternate and are situated laterally in the posterior third of the segment.

Head. The length of the head cannot be given, as it passes imperceptibly into the neck. The length from the anterior extremity to the proboscis bulbs is 1·5 mm. and its breadth is 0·4 mm. The two bothridia measure 0·5 mm. in length and 0·37 in breadth ; their margins are not thickened. The proboscides are armed with a large number of extremely small uniform hooklets. The neck is short, but varies in length.

Internal Anatomy. The muscular, excretory and nervous systems were not investigated.

Testes. These are very numerous and lie on each side of the median longitudinal axis until they are mature, when they completely fill the central field.

Vas deferens. The cirrus pouch is pyriform and extends almost to the centre of the segment; within it the vas deferens lies in several coils; it was impossible to decide, in whole mounts, whether the cirrus was armed or not.

Ovary. This is a very massive, bilobed organ situated posteriorly, and composed of acini densely crowded together; the vagina could not be traced in whole mounts.

Uterus. This appears very early and at first consists of a tube running in the median longitudinal axis of the segment. Its lateral walls become lobulated, and eventually it fills the segment and becomes full of eggs; the latter were immature and, in whole mounts, details relating to them could not be ascertained.

The worm agrees with Shipley and Hornell's description of this species except that it is larger, contains more segments, and these are not so elongated as in the type species. In all probability, Shipley and Hornell's specimen was immature and had been preserved in an elongated condition.

Rhynchobothrius binuncus, (Linton, 1909).

SYNONYM :—*Rhynchobothrium binuncum*, Linton, 1909.

One specimen of this worm was obtained by the author from the intestine of *Trygon* sp. (*walga ?*), Ceylon Pearl Banks, 27.11.10.

The species is distinguished by the peculiarly shaped hooks on the proboscides, by the worm being composed of about seven segments—the last being almost as large as the remainder of the worm,—and by the pore being situated in the posterior third of the segment.

Syndesmobothrium, Diesing, 1863.

SYNONYM :—*Symbothrium*, Diesing, 1850.

This genus is characterised by Diesing as follows :—

***Body** articulate, taeniaeform; neck tubular, rounded at the base; head tetragonal, with four terminal prominent bothria attached to head by posterior margin, cruciformly disposed, oval, slightly convex, joined with each other at the base by a membrane; proboscides four, filiform, armed, each one running through a bothrium (pedicel), excurrent at apex, long, retractile in the neck. Genital apertures marginal (?). In intestines of marine fishes of tropical America* (after Linton).

Syndesmobothrium rubromaculatum (Diesing, 1863) (fig. 11).

SYNONYMS :—*Tetrarhynchus rubromaculatus*, Diesing, 1863.
Tetrarhynchus platycephalus, Shipley and Hornell, 1906.

One specimen of what appears to be this species was collected by the author from the intestine of *Trygon kuhli*, Ceylon Pearl Banks, 1910. The proboscides were not protruded and it was therefore impossible to see the hooks properly. At least one longitudinal row appeared to point anteriorly. The worm is

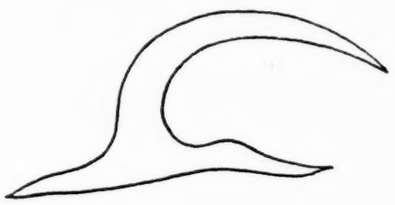


FIG. 11. *Syndesmobothrium rubromaculatum*. A hook, from proboscis. × 500.

composed of about ten segments, the last one being greatly elongated. The genital pore is situated in the posterior third of the segment. As the only specimen available was in poor condition, the general anatomy of the worm could not be made out. The species is somewhat closely related to *Rhynchobothrium benedeni* (Crety, 1890).

Otobothrium, Linton, 1890.

Body articulate, taeniaeform, head separated from body by a neck. Bothria two, opposite, lateral, each with two supplemental ciliated pits at the posterior free angles. Proboscides four, terminal, filiform, armed, retractile in neck. Reproductive apertures marginal (Linton).

Otobothrium magnum, n.sp. (figs. 12-14).

Three specimens collected by the author from the intestine of *Rhynchobatus djeddensis*, Pearl Banks, Ceylon, 25.4.1909.

EXTERNAL ANATOMY. The worms measure about 35 mm. in length and have a maximum breadth of about 0·5 mm. ; there are about fifty segments, the last one measuring from 2 to 3 mm. in length. The lateral margins are straight and the posterior margins are not salient ; the genital pores are irregularly alternate and are

situated on the lateral margin, in the posterior third of the segment. The neck is very short, measuring only 60μ in length.

Head. The entire head has a length of 4·5 mm. and a maximum breadth of 1·3 mm. ; the two bothridia each have a length of 1 mm. ; the proboscis sacs have a length of 1·75 mm. and are marked by fine diagonal lines crossing each other at right angles. The proboscis sheaths are spiral, and those portions lying posterior to the bothridia have a length of 1·75 mm. The proboscides are practically as long as the entire head, and are armed with hooks of various sizes and shapes arranged in horizontal rings. The hooks towards the tip are smaller

12 13

FIGS. 12 and 13. *Otobothrium magnum*, n.sp. Hooks from different parts of proboscis. × 250.

than the rest ; in each ring there are eight or nine hooks, the smallest hooks being situated in an antero-posterior line and the largest hooks on another antero-posterior line on the opposite side of the proboscis. Hooks of intermediate size and shape occur on the portions of the proboscides between these two lines.

INTERNAL ANATOMY. Owing to the material being scanty, the muscular, excretory and nervous systems were not investigated.

Testes. These first appear in about segment 32 and number about 300 ; their arrangement is peculiar in that about 50 testes lie posterior to the ovary and a separate cluster of about ten testes lie near the median extremity of the cirrus pouch. The remaining testes are distributed on each side of the middle line, in front of the ovary. The cirrus pouch was not fully developed and therefore

cannot be described; it appeared, however, to lie internal to the water vessels and to extend to the median antero-posterior axis.

Ovary. The ovary was rudimentary, bilobed, and situated a little distance from the posterior margin of the segment.

Uterus. This organ was also in a rudimentary condition and consisted of a slight irregular thickening running along the median antero-posterior axis of the segment.

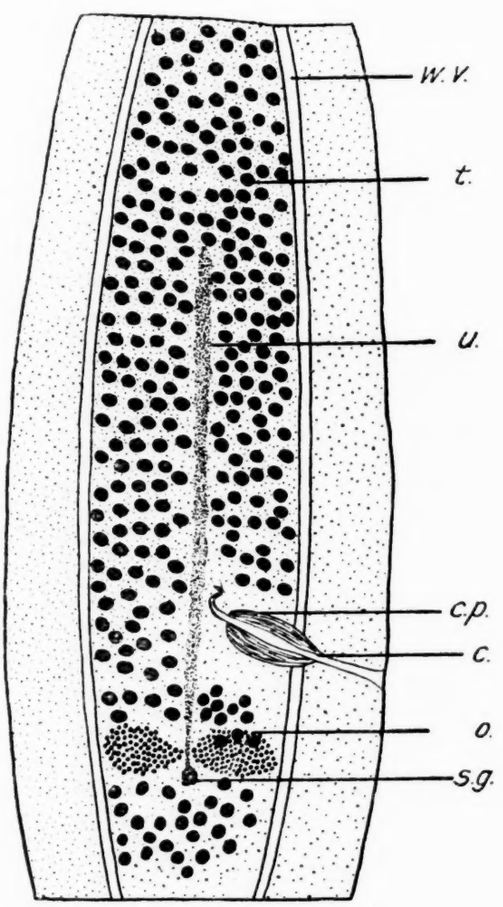


FIG. 14. *Otobothrium magnum*, n.sp. Mature segment. *w.v.*—water vessel; *t.*—testes; *u.*—uterus; *c.p.*—cirrus pouch; *c.*—cirrus; *o.*—ovary; *s.g.*—shell gland. × 112.

DIAGNOSIS. There are only four species belonging to this genus and they have all been described by Linton, viz.,

O. crenacolle, Linton, 1890 (described from an adult worm);

O. dipsacum, Linton, 1897 (described from a larva);

O. insigne, Linton, 1905 (described from a specimen not fully adult) ;

O. penetrans, Linton, 1907 (described from a larva).

O. crenacolle measures about 14 mm. in length, is composed of about twelve segments ; the genital pores are situated at the middle of the lateral margin of the segment.

O. insigne. In this species the genital pore is situated in front of the middle of the lateral margin of the segment ; the head terminates posteriorly in a ' collar ' which surrounds the anterior part of the strobila. The worm was not fully mature ; it measured 10 mm. in length and was made up of twelve segments.

O. penetrans. In this species the chief characteristic appears to be the fact that, posteriorly, the proboscis sacs are divergent.

O. dipsacum. The chief characteristics of this species (which, as noted above, was described from a larva) is the fact that each proboscis

' Has a longitudinal line towards which the short diagonal rows of hooks converge on each side. Near the base of the proboscides, where the hooks are somewhat scattered, from six to ten hooks to a row could be counted on each sac of the horizontal line under favourable circumstances. More than twice that number could be counted in the rows nearer the apex of the proboscis.'

O. magnum, n.sp., resembles *O. dipsacum* in having an antero-posterior line on each proboscis towards which the hooks gradually get smaller, but the number of rows of hooks is much less than that given for *O. dipsacum* and they differ also in shape. For these reasons the species is separated from all the species of *Otobothrium* hitherto described. It further differs from *O. crenacolle* and *O. insigne* in size and in the position of the genital pore. Type species are in the Liverpool School of Tropical Medicine.

Otobothrium dipsacum, Linton, 1897.

Several large larvae from the mesenteries of *Serranus undulosus*, South Silavaturia, Ceylon Pearl Banks. Collected by the author, April 2nd, 1909 ; also from *Diagramma crassispinum*, and *Balistes* spp., Ceylon Pearl Banks.

Genus *Coenomorphus*, Loennberg, 1889, emended.

With the characters of the family.

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PREFERENTIAL AND COMPULSORY BREEDING PLACES OF *AEDES (STEGOMYIA) AEGYPTI* AND THEIR LIMITS

BY

H. R. CARTER

ASSISTANT SURGEON-GENERAL U.S. PUBLIC HEALTH SERVICE

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Of course, the one absolute material requisite, material as distinguished from conditional requisites as temperature, etc., for the breeding of mosquitos of all kinds and in every place is *water*. Fruitful oviposition of this insect then in nature takes place only in relation to water; present at the time of oviposition for such as deposit their eggs only in water; or water in the future for such as deposit their eggs in places free from water, but which will be covered by it at the proper time for them to hatch.

Given water then, in *esse* or in *posse*, it is common knowledge that some species, even some genera, of mosquitos show decided choice in the nature of the places they select for oviposition and, where such places are available, use them to the exclusion of other collections of water, or at least so much more frequently that other places are not a serious factor in the propagation of this mosquito. Nevertheless, we sometimes find that when breeding-places of the preferred kind are *not* available, this mosquito will deposit eggs in other collections of water, although it may be not in all classes of such collections.

The class of breeding-places first mentioned—*i.e.*, those utilized for oviposition when all kinds are available, we may call the *preferential breeding-places*, or the *breeding-places of election*, for the mosquito in question. The second kind—*i.e.*, those in which

eggs are deposited when, and only when, breeding-places of election are not available, may be as properly called *compulsory breeding-places* and naturally the places in which, in nature, eggs are not deposited even when they are the only collections of water available, are beyond the natural limits of even compulsory breeding-places and from the last two we may be able to determine the limits of breeding for this mosquito in nature.

Note here that when we speak of oviposition or of breeding in any place or class of place, we mean oviposition or breeding sufficient to be a serious factor in the propagation of this mosquito. When less than this it is considered negative. This is, of course, rather the view-point of the sanitarian than that of the entomologist, yet for the sanitarian it is quite consistent. In *Anopheles* surveys we grade breeding-places as 'of sanitary importance' and 'of no sanitary importance,' and disregard the latter. In yellow fever campaigns we count our work complete when the production of *Stegomyia* is so reduced that yellow fever cannot be kept up by them, although a minimal breeding may still be going on. So the occasional finding of a few larvae of a certain mosquito in places usually free from them does not justify our putting such places in the same class with those in which larvae are habitually found in numbers. In sanitary matters, and I confess that I am inclined to look at mosquitos from a sanitary standpoint, no one cares about *a* mosquito. It is only when zeros accumulate behind them and they show as hundreds, thousands, or ten thousands that they become important—to the sanitarian, I mean.

What we have said of the relation of oviposition to the different classes of possible breeding-places may be true of all kinds of mosquitos ; it is true of many kinds, but I am confining myself here and now to one : *Aedes* (*Stegomyia*) *aegypti**—the known vector of yellow fever and dengue. I had purposed to take up the same question about some species of *Anopheles*, malaria vectors, but this would have made this article entirely too long,—a book not a paper.

For this mosquito, it is a matter of common knowledge that its breeding-places of election are collections of water in artificial (man-made) containers, or things that simulate them : wood, cement, stone and brick being preferred to metals as the material for such

* = *Stegomyia fasciata*, Fabr. Editors.

containers and wood preferred to cement. Calabashes have seemed to be especially attractive to them. Yet long ago my attention was called to the fact that these conditions of breeding, although usual, were not obligatory and, if lacking, could be substituted by others. I was in Panama City in the last part of November or very early in December of 1905, and was there inspecting the *Stegomyia* work. The man immediately in charge was a very capable one, and we visited together a number of premises, houses and patios. In all, the *depositos* of water kept for domestic use were protected against access of mosquitos. Cisterns, *tinajas* and *ollas*, all were either empty or covered, some with wire-netting, some with cloth tied on and some with well-fitting, solid covers; no water in any of them was accessible to mosquitos. Of a considerable number of premises he had inspected before I came, he reported all as being negative for larvae and in none that I examined with him could I find larvae or pupa-casts floating on the surface ; in large collections of water the latter show up far more readily than larvae themselves.

Yet in one place I was able to show him *Stegomyia* larvae, possibly ten or a dozen, and maybe twice as many, taken with a tea-spoon from a rot-hole in a tree, small ones, in the little collections of water in the axils of the leaves of some ornamental plants in the patio—a species of Colocasia. Guessing the cause for the oviposition in these places, I directed him to place, and assisted him in placing, a number of calabashes, *ollas*, and other containers of water in shady and partly dark places about the house and patio, with instructions to empty them every Saturday at the weekly inspection. The result was successful: not only was this patio free from larvae for the next three or four Saturdays when I inspected it, but I am persuaded that neither this inspector nor any of his men ever failed to examine and to provide for the elimination from any other patio of all such breeding-places as those in which we had found these larvae.

As we had found practically all of the *depositos* of water—the preferential breeding-places of this mosquito—inaccessible to it in the vicinity in which we had found the larvae just mentioned, and had not found larvae in rot-holes in trees or the axils of Colocasia leaves elsewhere, it is fair to believe that the places in which larvae were found in this patio were breeding-places of compulsion, used

only because none more suitable were available and this seemed to be, to an extent, confirmed by the result of making suitable breeding-places accessible.

It may be noted here that while we had already occasionally found *Stegomyia* larvae in the small collections of water in the axils of the ornamental Colocasias, yet we had come to regard them as of minor sanitary importance, as so small a proportion of them developed into imagos. This, indeed, seemed to be generally true of the compulsory breeding-places of this mosquito. Her progeny seemed to have a much better chance of reaching maturity in the breeding-places of election than in those she utilized only when these were not to be had. This may not be without exception, but the one exception which I have seen reported involves an artificial condition and is not pertinent to our subject.

What are the limits to the compulsory breeding-places of this mosquito ? On the Isthmus of Panama we did not find its larvae in marshes nor in seepage out-crops—so favourite a place for *Anopheles*—nor in sluggish streams and never in a street gutter, nor a roadside puddle, except when we were reasonably sure that they had been washed in from some other place, as from an overflowing cistern or a sagging house-gutter, and this was true whether more suitable breeding-places were available or not. In other words, such places were beyond the natural limits of breeding—complete breeding, from oviposition to imago—of this mosquito on the Isthmus of Panama.

Is this true everywhere, or, as happens sometimes with other mosquitos, is there a regional variant to this part of its biology ? Some years ago Francis, of the U.S. Public Health Service, as a result of his observations for some three years in and about Mobile, Ala., stated that ' larvae of this mosquito have not been found in any collection of water the bottom of which was of mud.' As intended, ' not in mud-puddles,' this seems correct and would be so accepted, I think, by all of you. As expressed, it doubtless was correct, anyway. The implication, however, that this was a general rule and that the mud bottom of a collection of water was the factor which determined that *Stegomyia* should not breed therein is erroneous. During 1920 and 1921 in Payta, Sullana, Piura, Catacaos, and in practically every other town in Northern Peru

which we examined, we found *Stegomyia* breeding and breeding freely, in the great *botijas*, or *tinajones*, used to store water in that country, although their bottoms were generally covered with mud, from six to ten inches deep. Very evidently, mud at the bottom of the water in no wise prevented their breeding in it.

Admitting that we have not found them breeding completely in mud-puddles, what are the factors which prevent this ? I think we can name one of them, and possibly the determining one. At Catacaos, a town of about 40,000 people in Northern Peru, where we had yellow fever in 1919 and 1920, there were many wells, say from 100 to 200. They were merely holes dug in the ground from six to twelve feet deep according to the depth of the ground water. We never found *Stegomyia* larvae in these wells, even when breeding was fairly well-controlled in the artificial containers in their neighbourhoods. Plenty of larvae of other mosquitos, but none of *Stegomyia*. At Casa Grande, also in Northern Peru but further south, in every house of a skilled employee there was a well and we found *Stegomyia* larvae in exactly 100 per cent. of these wells ! Why this difference ? Could it be because the wells at Casa Grande were in houses occupied by men while those at Catacaos were in the open ? It did not seem so, because the wells for general service at Casa Grande were out of doors and all that we examined, some eight or ten, showed *Stegomyia* larvae, although fewer than those in the houses. There was another difference between the wells at the two places besides their locations in, and not in, houses. Those at Casa Grande were lined with brick and cement, while those at Catacaos were unlined, simply dug in the clay. In Chiclayo, too, situated between the two places, there were a number of wells, some lined and some unlined. In none of those unlined which we examined did we find *Stegomyia* larvae ; in none of those lined, whether with wood or with brick and cement, did we fail to find them !

Really this explanation had been anticipated. Anyone who has seen the oviposition of this mosquito standing just at the edge of the water, sometimes on the water, sometimes on the container, sometimes partly on both water and container, would naturally guess that the physical nature of the sides of the container *at the water's edge* might well be a factor in her choice of a place for oviposition ; while, unless it affected the quality of the whole body of water, the nature of the bottom would be unknown to her.

After considerably more observation on this point in Peru by Hanson and by Dunn and their men and much inquiry, verbal and by letter, of men doing *Stegomyia* control work in other countries : Mexico, Yucatan, Central America, Panama and Colombia, I felt myself justified in presenting this formula, modelled on that of Francis, as limiting the breeding-places of this mosquito :

' We have not found this mosquito (Aedes (Stegomyia) aegypti) breeding, in nature, completely, that is, from oviposition to imago, in any collection of water, all the sides of which at the water's edge were of mud.'

It was thus given out in a lecture to the Laboratory Class of Officers of the U.S. Public Health Service in Washington in the Spring of 1923, and, a little later, to the class at the School of Hygiene and Public Health of The Johns Hopkins University in Baltimore. As expressed it was correct ; neither myself nor anyone of whom I had inquired had ever seen *Stegomyia* breeding in the places excluded by this formula. Even the implication was logical and I then thought correct, yet I have recently learned that, taken as a universal formula, it is not.

Last September I received, through the International Health Board of the Rockefeller Foundation, copies of official reports sent them from the British Colonial Office of the yellow fever epidemics of 1922 and 1923 in British West Africa. Among these epidemics was one at Salt Pond on the Gold Coast, small but virulent, as African yellow fever has usually proved to be. In the report on this it was stated that the principal source of *Stegomyia aegypti* at this place was the lagoon and the tracks in the mud around it of the men and boys who go bathing there. This production was stated to be large and to be uncontrollable except by a major engineering project. As illustrating the amount of this breeding, the reporter stated that in one collection of water (in this mud) of about ten inches square, characterized by him as ' a typical *Anopheles* breeding-place,' the larvae were so abundant that ' they nearly filled the saucer in which they were dipped up ' and it was added that these larvae developed into imagos of *Stegomyia aegypti* and *Culex fatigans.*

The writer of this report, Dr. Lorena, and his immediate superior, Dr. Watt, both officials of the Sanitary Service of the Colony, ascribed this breeding ' in such unusual places ' to the complete

elimination of the usual breeding-places from the town and its environment.* This, of course, would make the breeding of *Stegomyia aegypti* in and about the lagoon a compulsory breeding, there being no more suitable place available. Yet there are other reports, previous to the one quoted, implicitly accusing the Salt Pond Lagoon and its environment of producing *Stegomyia aegypti* normally—*i.e.*, when there had been no interference with their normal breeding-places. That of Drs. Horn and Tytler (Oct. 16, 1920), made in connection with the Yellow Fever Commission of the International Health Board of the Rockefeller Foundation of 1920—the one on which Gorgas was serving when he died—implies the production of *Stegomyia* in and about this lagoon, as it suggests the canalization of this lagoon as affecting the prevalence of yellow fever in Salt Pond. It also states that the same conditions exist at Seccondee and other places on this coast as at Salt Pond. In some of the reports, too, of the British West African Yellow Fever Commission—the last I saw were, I think, of 1913—and the papers transmitted with them, there were also recommendations for the abolition of this lagoon and cure of the mosquito breeding conditions around it, on account of their effect on the propagation of yellow fever at Salt Pond.

Be this as it may, neither Le Prince nor myself nor any one of the many whom I have consulted have ever seen, in the Americas, *Stegomyia* breeding, from oviposition to imagos, in mud-puddles. Nor have either of us even seen this mosquito breeding anywhere in anything like the profusion implied in this report. *Culex fatigans*, yes; *Stegomyia*, never. It is a pity the reporter did not give the proportion of the two kinds of imagos developed from his pool in the mud.

Nevertheless we cannot, on this account, reject this report as erroneous. I have seen too much of what, I suppose, may be called 'regional variation in the biology of mosquitos' to refuse credence to a well-attested statement about them, as I consider this to be, simply because it is not in accordance with my own observations made in an entirely different region. And, while I would greatly like to examine for myself the breeding of this mosquito in and

* Why, knowing this, the 'usual breeding-places' in the town and its vicinity were not at once re-established one cannot imagine. Knowing where they were these could have been easily controlled; the breeding about the lagoon had been reported as 'uncontrollable.'

about this lagoon at Salt Pond, yet I feel that we must accept the statements of fact as given in the report quoted. Without denying it I am, however, less inclined to accept the breeding of *Stegomyia aegypti* in and about the lagoon at Salt Pond *as its normal breeding there—i.e.*, as taking place when water in artificial containers was available to them for oviposition, because (1) the evidence for it that I have seen is implied rather than direct and is less in detail, (2) it seems to me antecedently more improbable, and (3) the characterization of these places, the lagoon and the tracks around it, in Lorena's report, as 'unusual breeding-places' for this mosquito, used only because the usual ones in the neighbourhood had been eliminated.*

In any case, however, we must change the formula just given you by limiting it to the Americas. And I am entirely willing to do this because all that I know of *Stegomyia* breeding at first-hand is its breeding in the Americas. Our formula should then read:

'*In the Americas* we have not found this mosquito, *Aedes* (*Stegomyia*) *aegypti*, breeding, in nature, completely, from oviposition to imago, in any collection of water all the sides of which at the water's edge were of mud.'

This is not only correct as expressed, but its implication that collections of water in the Americas under the conditions specified are not found breeding *Stegomyia* in nature is, I am inclined to think, correct also. For, since the receipt of the West African reports mentioned, I have been in communication, in reference to the breeding of *Stegomyia aegypti* in puddles, with a number of men whom I know and who are working, or have been working, in the Americas, for the elimination of yellow fever: with White, Scannell, Walcott and others in Brazil; with Hanson and Dunn, an entomologist, in Colombia; with Caldwell, Connor, Scannell and Houle in Mexico and Yucatan and with Connor in the Guianas, and no one has, so far, reported having found *Stegomyia* breeding in nature completely in mud-puddles and a number report directly to the contrary. I think then that, for the present at

* After the reading of this paper the writer had opportunity to talk to Dr. A. E. Horn, to whose report of 1920, made jointly by himself and Dr. Tytler, allusion has been made. Dr. Horn stated that he had not seen larvae of *Stegomyia aegypti* in the Lagoon at Salt Pond, nor in the puddles adjacent thereto, nor had he seen them in similar places at Seccondee. The inference then drawn from the Report of Drs. Horn and Tytler, although logical, was incorrect.

least, the implication of our modified formula, that complete breeding of *Stegomyia* in mud-puddles does not, in nature, occur in the Americas may be accepted. It is supported by a considerable amount of evidence ; negative evidence, indeed, but from the nature of the question no other kind is here possible. Is it enough—negative evidence is convincing in proportion to its mass—to justify us in asserting that, in the Americas, mud-puddles are beyond the limits of natural *Stegomyia* breeding—even of natural compulsory breeding ? I think so, but we should know when the work in Brazil is finished.

At any rate, there seems to be a difference in the limits of the natural compulsory breeding-places of this mosquito in the Americas and at Salt Pond and, it may be, in other places in West Africa. Possibly the biology of the African strain is less rigid than is that of ours ; that it can adapt itself to conditions which the American strain is unable to meet. Well was it for us that this last was true ! Had the Salada and all the puddles about Guayaquil produced *Stegomyia*, the elimination of yellow fever from that city had been more difficult even than it proved. So if the conditions of breeding as reported at Salt Pond are general in West Africa, the elimination of yellow fever therefrom will be decidedly more difficult than if the limits of the breeding of this mosquito, both normal and compulsory, were there the same as we have found them to be in the Americas.

Very obviously, then, the utilization by *Stegomyia aegypti* of breeding-places other than those of election is of decided importance to the sanitarian. It makes its control more difficult than if only the latter were used. If, as at Salt Pond, one must control its production not only from artificial containers in the town, but from mud-holes and ponds as well, the difficulty may be many-fold greater than if the first only were involved. And this difficulty might be *very* greatly increased if this adaptability of the insect to breeding in what are now abnormal places should prove to be a characteristic which can be cultivated and increased by practice, as has apparently happened with at least one characteristic of *Anopheles maculipennis*.*

It would seem advisable then so to plan our methods of *Stegomyia* control, as to limit as much as possible the use of compulsory breeding-places by this mosquito. This depends on the facilities

* The development of a preference for the blood of domestic animals over that of man in these mosquitos in certain parts of France and Denmark.

available for oviposition. As long as the mosquito has easy access to a sufficiency of breeding-places of election, she will not seek to deposit eggs in any other. *It may be advisable then to provide her with such breeding-places of election.*

Does to provide suitable breeding-places for, and easy of access to, this mosquito, of which we are trying to rid ourselves, seem a paradox? I have been asked (about *Anopheles*, however) if I 'wished to encourage them to breed?' Not exactly, but we don't care whether they breed or not, *provided there be no production.* Eggs, larvae and pupae are absolutely innocent, and if breeding is stopped short of their final development into the imago, which alone is offensive, we are satisfied. Eggs and larvae then, as many as you wish (pupae are too close to the final change to risk) provided there is no development of imagos.

Control of this mosquito then* by measures which allow of oviposition in their preferential breeding-places may be, in certain places will be, the preferential method to adopt.

Now by the use of fish, by oiling (not usually a method of election), and by emptying and refilling water containers at proper intervals of time, we can, in general, sufficiently control the production of *Stegomyia* and yet allow them access to their preferential breeding-places for oviposition. These methods then involve no risk of driving them to unusual breeding-places, the control of which may be difficult. Nay, even the removal of containers and the covering them so as to exclude access of mosquitos, methods very advisable for permanent work, can be used if other suitable breeding-places be provided accessible to them. The production of imagos from these breeding-places provided can be controlled, sometimes by fish or, as we know where we placed them, easily and absolutely by emptying them at the weekly inspection

It may be worth mentioning to you that a small amount of sugar added to the water is reported by Fielding to increase its attraction to this mosquito for oviposition, although her progeny do not thrive on it: an instance, and they are rare among the lower forms of life, in which the maternal instinct is at fault.

Very obviously, then, before systematic work against yellow fever is begun in West Africa a survey must be made to determine the

* This is a general rule applying to *Anopheles* no less than to *Stegomyia aegypti.*

limits there of both the normal and the compulsory breeding-places of its yellow fever vector, or vectors. If the compulsory breeding-places be beyond our ready control, we should, by some of the methods just given, try to arrange for the control of normal breeding, so as to produce little or no compulsory breeding, remembering always that less than a 100 per cent. reduction of *Stegomyia* will eliminate yellow fever.

If the normal breeding habits of this mosquito in West Africa generally are those implied by the report of Horn and Tytler and similar reports as obtaining at Salt Pond and Seccondee,* the elimination of yellow fever from that region would be, I think, impracticable by the methods we have used in the Americas.†

* Sir Rubert Boyce also notes having seen *Stegomyia* breeding in a pool on the mud-covered roof of a hut (*i.e.* 'puddle breeding') in West Africa. I think he does not state where.

† See footnote: page 500.

TWO NEW SPECIES OF REPTILIAN CESTODES

BY

PROFESSOR O. FUHRMANN, PH.D.
(NEUCHÂTEL, SWITZERLAND).

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[These two new species were sent to us for determination by Dr. H. O. Mönnig, Research Officer, Onderssteppoort, Pretoria, to whom we here tender our thanks.]

OOCHORISTICA THEILERI n.sp.

Host : *Agama hispida?* Locality : Pretoria.

There are altogether forty specimens of this interesting worm, collected from a Lizard, which Dr. Mönnig believes to be *Agama hispida*, var. *distans*. With the exception of a single specimen to which we will refer later, all the mature specimens, *i.e.* specimens possessing egg-capsules containing embryos, are 4-8 mm. in length, with a maximum width of 0·85 mm. The scolex measures 0·3-0·4 mm. across the suckers, the latter measuring 0·14 mm. in diameter. The scolex is not separated from the strobila by any stricture or neck-like structure, and the latter maintains a uniform width of 0·76 to 0·85 mm. throughout its entire length. The segments are indistinctly separated from one another, and are all, excepting the last ones, wider than long.

The *Musculature* of the strobila is of special interest. Whereas the *Oochoristica* spp. usually possess two or three layers of longitudinal muscles, our species only possesses a single layer of bundles made up of longitudinal muscle fibres. A similar disposition is to be found in *O. zonuri* Baylis, only with this difference : that the latter species possesses 40 dorsal and ventral bundles made up of 15 to 20 fibres each, whereas *O. Theileri* possesses the weakest musculature that we have ever seen. There are about 25 bundles made up of 2 to 4, exceptionally of 5 to 6 longitudinal fibres. As these bundles are spindle-shaped, it often happens that on transverse sections there are only to be seen a few irregular bundles, between which

appear isolated longitudinal fibres. The transverse muscles are weakly developed, and the dorso-ventral ones very fine. Calcareous corpuscles are scarce.

The *Excretory System*. This presents the typical disposition of four winding vessels of which the two ventral ones are united by a fine network of secondary vessels. We have also noticed other ramifications, but have been unable to follow them up, owing to their minuteness.

The *Genital Organs* appear close behind the scolex, and develop very uniformly in all the specimens that we have examined. The male and female gonads attain their sexual maturity in the fifteenth to the eighteenth segment. They first of all develop slowly, then suddenly from the twelfth to the fourteenth segment onward they develop very rapidly. In the sixteenth to the nineteenth segment the ovary has almost entirely disappeared, and the egg-capsules have begun to form ; the formation of the latter is terminated in the next five to six segments, then follow a small number of gravid segments (see fig. 1 B). The strobila are thus constituted according to their length, of 23 to 28 segments. The genital pores alternate irregularly, and open into a remarkably deep genital atrium, situated slightly anterior to the middle of the border of the segment. The genital atrium (see fig. 1) is as large if not larger than the cirrus pouch, and presents two peculiarities, namely, it is lined with a cuticula armed with minute spines, and beneath which there is to be found a distinct layer of sub-cuticular cells from which arise a radiating musculature which is lost in the parenchyma.

The bottle-shaped cirrus pouch is 0·12 mm. long, and has a very muscular neck, although the distal extremity possesses but a weak muscular wall. When evaginated, the stout *cirrus* is 0·1 mm. long and measures at its base 0·04 mm. in diameter. The basal portion of the cirrus is covered with the same spines as those lining the genital atrium. The cirrus and *ductus ejaculatorius* are surrounded by a sheath of muscles. There is a well-developed retractor muscle attached to the cirrus pouch. The *vas deferens* presents a few loose coils. The *testes*, 26 to 30 in number, are entirely situated behind the female gonads, and form two groups, which may occasionally meet in the mid-line when the segments are much extended. This disposition has as yet never been recorded from *Oochoristica* spp.

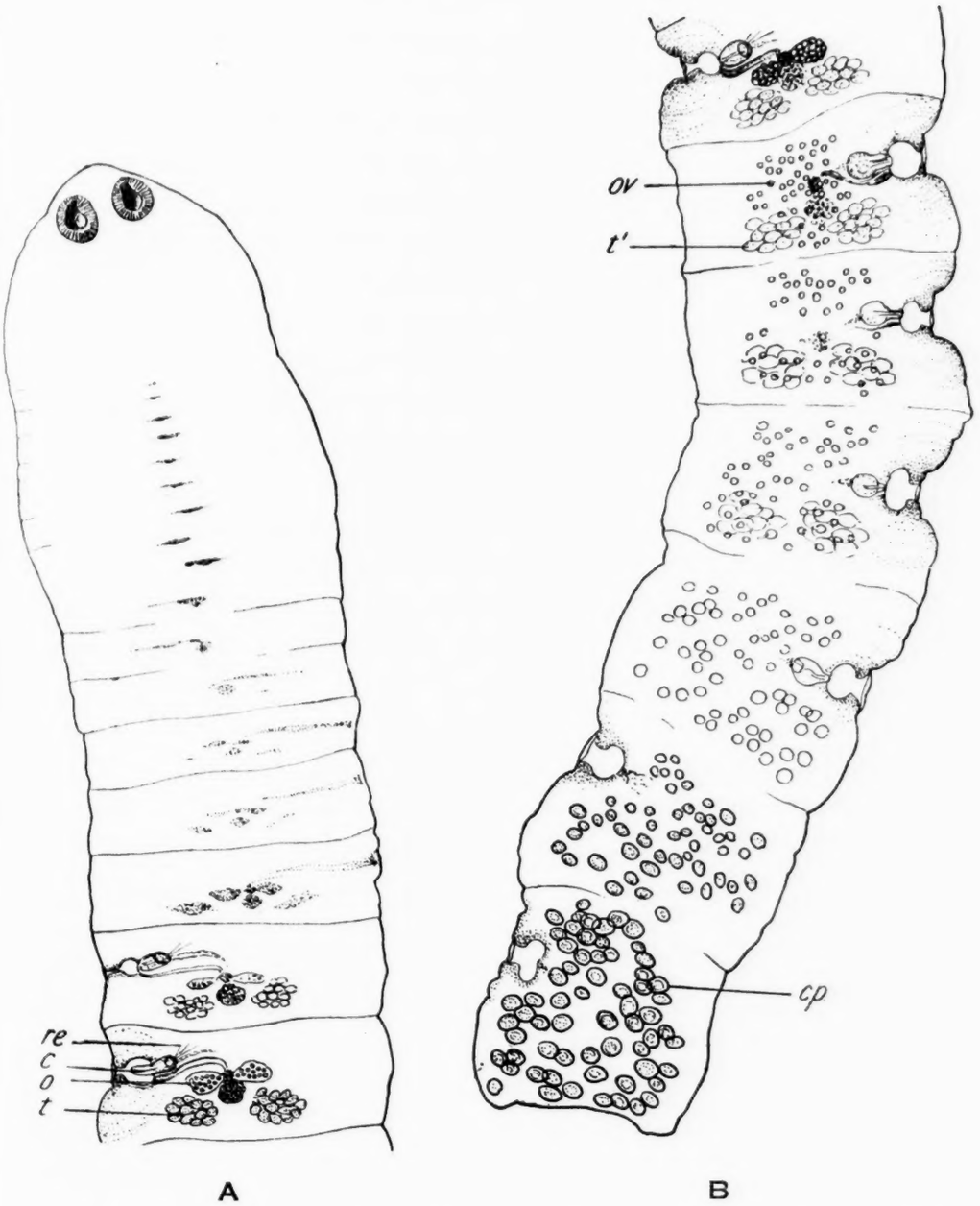

FIG. 1 A and B. *Oochoristica Theileri* n.sp. Mount of an entire worm. *c.*—cirrus; *cp.*—egg-capsules; *o.*—ovary; *ov.*—ova dispersed in the parenchyma; *re.*—retractor muscle of the cirrus pouch; *t.*—testes; t^1.—testes in regression.

As is usually the case in this genus, the *vagina* opens behind and on the same level as the cirrus pouch, and presents in its proximal portion a region as long as the cirrus pouch, possessing a thick muscular wall, and of the same diameter as the cirrus ; the vagina then forms a narrow tube which dilates near the ovary to form a small *receptaculum seminis*. The *ovary* is slightly displaced towards the poral side of the segment, and attains a maximum width of 0·2 to 0·24 mm. It consists of two wings, oval in shape, and not lobed ; the poral wing is slightly smaller than the aporal one. The yolk gland is almost spherical, and measures 0·08 to 0·1 mm. in diameter. The shell gland is situated dorsally, and is 0·06 mm. in diameter ; it surrounds a very distinct ootype.

We have already remarked on the fact that both the male and female genitalia attain their sexual maturity in the same segment ; this is, nevertheless, contrary to what occurs in most Cestodes. The testes, however, still persist in several segments, whereas the ovary and the yolk gland disappear suddenly from one segment to the next. In mature segments, there appears between the male and female genitalia a sac-like uterus, which in the next segment has formed out-pocketings, especially anteriorly. These diverticula are filled with ova ; in the next segment, however, they form as many capsules as there are ova, each ovum being thus enclosed within a capsule. The segmentation which begins as soon as the ova enter the uterus, continues its course, and four segments further on, *i.e.* six segments posterior to the mature female gonads, the egg-capsules are definitely formed. These capsules can be easily distinguished, as they stain very deeply ; they seem to be formed of a hard and fragile substance, because on sections the capsules are broken into polygonal pieces showing a clean fracture. First situated in the field between the excretory vessels, the capsules partly push their way beyond the latter, and even beyond the nerve stems. The capsules are 0·06 mm. in diameter, and the embryos, surrounded by a very thin membrane, measure 0·03 to 0·04 mm. in diameter.

Whereas the number of ova contained within the ovary exceeds 200, we have never found more than 100 egg-capsules, each containing a single egg. One must, therefore, admit that when the ovary disappears suddenly, more than half the ova are unfertilized, and are resorbed. There are from 60 to 100 capsules per segment. On

several occasions we have found in a single strobila segments containing only 20 capsules, along with about 70 very small capsules presenting the aspect of calcareous corpuscles, and which are nothing else than aborted embryos, the abortion being due to inadequate fertilization.

As we have already mentioned above, our material contains a much larger specimen possessing the same anatomy. This species, *O. Theileri forma major*, presents, however, certain peculiarities with regard to the development of the sexual organs, and to the number of uterine capsules.

The specimen is 30 mm. long and has a uniform width of 1·7 mm. The *scolex* measures 0·5 mm. across the suckers ; the latter are small and only measure 0·14 mm. in diameter. Immediately behind the suckers, the scolex widens out to attain the width of the anterior part of the strobila.

The *Sexual Organs*, and especially the female gonads, attain their maturity only in the forty-fifth segment, whereas in the other species examined they were already mature in the sixteenth or seventeenth segment. In the forty-sixth segment the female gonads have entirely disappeared, and the ova are surrounded by the uterine capsules, which attain their full development in the seventieth segment. The last thirty-one segments all contain fully-developed capsules. With regard to the number of capsules, we find it to be twice as great as that for the small species ; this may perhaps be explained by the fact that the segments are twice as long as those of the other species.

There is no doubt that these two forms belong to the same species, and such differences undoubtedly arise from the fact that when the young Cestode arrives in the intestine of its host, it immediately developes its genital organs and ova ; later on this development slows, and the development of the genitalia and of the ova takes place much more gradually, *i.e.* occupies a much larger number of segments. Probably both these forms belong to two different infections.

Of the twenty-six species of *Oochoristica* known, seven are found in Africa, four occurring in Reptiles; these are: *O. zonuri*, Baylis, *O. agamae*, Baylis, *O. truncata* (Krabbe), and *O. crassiceps*, Baylis, all different from *O. Theileri* n.sp.

OPHIOTAENIA MÖNNIGI n.sp.

Host : *Leptodira hotambeia*. Locality : Pretoria.

This typical Proteocephalid was collected from the intestine of a red-lipped Snake *Leptodira hotambeia*.

The scolex of this only specimen is missing, but the anatomy is sufficiently characteristic to create a new species, for which we propose the above name. This small worm probably does not exceed 50 mm. in length, the greatest width being 1·8 mm. The youngest proglottids are 0·09 mm. long and 0·78 mm. wide, the next segments, showing the genital primordalia, are 0·17 mm. long and 0·9 mm. wide, and the segments in which the genital organs have attained their full development measure 0·79 mm. in length and 0·9 mm. in width. The gravid segments, on the other hand, measure 2·5 mm. in length and 1·5 to 1·8 mm. in width. The segmentation of the strobila is indistinct, as is the case for all *Ophiotaenia* spp.

The *Muscular System* is chiefly formed of longitudinal muscles, whereas transverse and dorso-ventral fibres are scarce. In young proglottids in which the genital primordalia have not yet appeared, the longitudinal muscles form a circular layer 0·04 mm. wide, surrounding the medullary parenchyma : the latter is only 0·03 mm. wide and contains numerous transverse fibres, dorso-ventral fibres being very scarce. We have found occasional calcareous corpuscles. In gravid segments the longitudinal muscle layer is displaced towards the periphery of the segment because the medullary parenchyma is almost entirely occupied by the uterus, which increases the width of the latter by nearly five times. In these segments the longitudinal muscle fibres form a narrower and more irregular layer. Transverse and dorso-ventral fibres are exceedingly scarce.

Excretory System.—Two pairs of longitudinal excretory vessels are to be found throughout the entire strobila. The ventral vessels are thin-walled, and measure from 0·03 to 0·04 mm. in diameter. We have been unable to find commissurae nor have we seen any ramifications ; the latter must be scarce, contrary to what is found in the other species of *Ophiotaenia*. The dorsal vessels, 0·008 to 0·012 mm. in diameter, are to be found dorsal to the ventral vessels in the anterior part of the anterior region of the strobila, whereas in the posterior region the former are displaced towards the exterior (see

fig. 2 A). The walls of the dorsal are thicker than those of the ventral vessels and are surrounded by a layer of plasma containing occasional nuclei. In the anterior region of the strobila these vessels are surrounded by a single layer of stout longitudinal muscle fibres, the latter being derived from the longitudinal musculature. We have been unable to study the *foramina secunda* for want of material.

Nervous System.—The two longitudinal nerve stems, 0·03 mm. in diameter, are situated close to the lateral borders of the segment, and in places touch the sub-cuticular cell layer. The two accessory nerves, the one dorsal and the other ventral to the main nerve, are exceptionally distinct in the anterior part of the strobila.

The *Genitalia* develop slowly, and open into a fairly deep genital atrium, which alternates irregularly, and which is situated about the middle or slightly posterior to the middle of the border of the segment.

The male genitalia consist of a relatively small egg-shaped *cirrus pouch* with weak muscular walls. The distal portion of the pouch, contrary to what occurs in most of the *Ophiotaenia* spp., does not reach as far as the ventral excretory vessel. The total length of the pouch is 0·2 mm., and the diameter is 0·1 mm. It contains a large muscular cirrus. The coils of the *vas deferens*, situated outside the cirrus pouch and between the latter and the median stem of the uterus, are in places greatly dilated and can attain a diameter even greater than that of the cirrus pouch (0·1 to 0·12 mm.). This portion of the *vas deferens* is at least five to seven times greater than the cirrus pouch, and replaces functionally a *vesicula seminis* which is lacking. Within the cirrus pouch the *vas deferens* may also be dilated, but never forming a distinct *vesicula seminis*. The *testes* occupy two narrow dorsal bands 0·17 mm. wide, situated in the lateral fields of the segment. In mature segments the testes number about 80 and measure from 0·05 to 0·07 mm. in diameter. They are situated to the right and to the left of the dorsal excretory vessels. In gravid segments in which the vessels are displaced, as already stated, they are situated inside of the latter. The *vasa efferentia* form a fine network.

The *Vagina* opens anterior to the cirrus pouch, and presents a slight *sphincter vaginae*; there is no distinct *receptaculum seminis*. The *ovary* forms in the posterior part of the segment a narrow band

just passing beyond the ventral excretory vessels. The ova possess very large nuclei nearly touching one another, so that the ovular plasma does not seem to be very abundant. The ovicapt is hardly developed, the shell gland is median, and is situated dorsally to the ovary. The *vitellaria* occupy two lateral bands situated within the

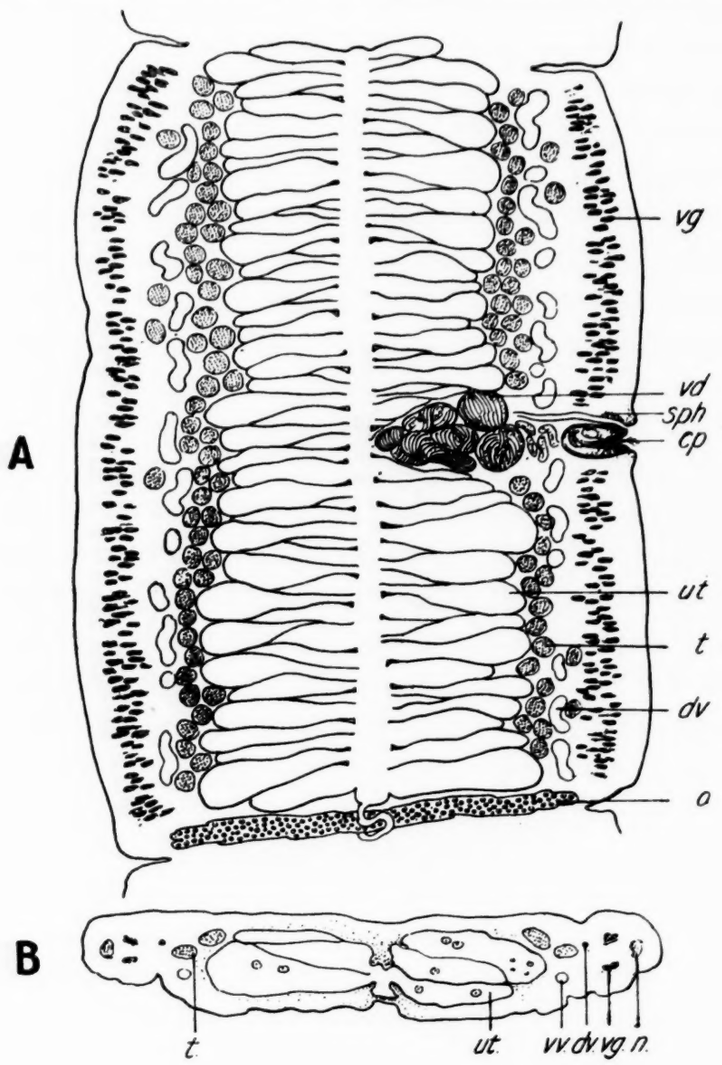

FIG. 2 A and B. *Ophiotaenia mönnigi* n.sp. A. Horizontal section of a mature segment. *cp.*—cirrus pouch; *dv.*—dorsal excretory vessel; *o.*—ovary; *sph.*—sphincter vaginae; *t.*—testes; *ut.*—uterus; *vd.*—vas deferens; *vg.*—vitelleria. B. Transverse section of mature proglottis. *n.*—longitudinal; *v.v.*—ventral excretory vessel. Other letters as A.

longitudinal nerve stems. The largest follicles are 0·12 mm. wide and 0·03 mm. long measured lengthways with regard to the segment. On transverse sections there are usually to be seen one dorsal and one ventral follicle (see fig. 2 B). The *uterus* is of the typical shape :

from a median stem fifty to fifty-seven diverticula, which are much longer than those of the other *Ophiotaenia* spp. (see fig. 2A), branch off on either side. On transverse sections we were able to observe that it is not a single diverticulum that branches off from the median stem, but at least two, and exceptionally three, diverticula all situated in the same dorso-ventral plane. Therefore, the number of diverticula indicated should be at least twice as great. We were unable to observe any uterine pores; we have, however, observed on the ventral surface of the segments, a certain number of short, irregularly placed diverticula arising from the median stem, and reaching as far as the cuticula, where probably a rupture of the tissues allow the ova to escape. The embryos measure 0·012 to 0·013 mm. in diameter, and are surrounded by two envelopes, of which the exterior and thicker one is 0·03 mm. in diameter.

Our new species, although very characteristic, reminds one of the other South African species placed by Rudin (1917) in the *O. Theileri* group. This group contains the following species: *O. Theileri*, Rudin (South Africa), *O. Zschokkei*, Rudin (South Africa), both found in *Naja* spp., and *O. adiposa*, Rudin (Cameroon), and *O. gabonica*, Beddard (Gaboon) from *Bitis* spp.

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ON A COLLECTION OF LINGUATULIDS IN THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

BY

T. SOUTHWELL

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The only feature of interest in this collection is the occurrence of several species of Linguatulids in fresh hosts and in new localities, the most important being the presence of *Porocephalus clavatus* in African snakes.

An attempt had been made to provide a key for all the known genera and species, but in the absence of adequate descriptions of numerous species the result is somewhat unsatisfactory. The keys are compiled from the descriptions given by Shipley and by Sambon in their respective papers, and the definitions of the various genera, sections, etc., are after Sambon. The only species I have been able to examine are pointed out in the text.

Except where otherwise stated, the measurements given refer to females only.

The Linguatulids are parasitic worm-like animals which are annulated in such a way that they resemble segmented worms. The annulations are, however, purely superficial for there is no segmentation of the genital organs such as exist in the Cestoda.

The systematic position of this family of parasites in the animal world is a matter on which some doubts exist, but the generally accepted opinion is that they are an aberrant, or degenerate branch of the Phylum Arthropoda and of the class ARACHNIDA (scorpions, spiders, etc.) and of the Order **Acarina** (mites and ticks) *i.e.*, that they are more or less related to ticks and mites, even though superficially they are worm-like in appearance. The limbs are represented by four hooks which encircle the mouth ; the muscle fibres are striated. Respiratory, circulatory and special sense organs are absent. The gut is a simple tube with no appendages ; the sexes are separate as in other Acarines and there is a small nerve ring which surrounds the oesophagus.

The synonymy of the parasites included in this family is very extensive. Frölich, in 1789, erected the genus *Linguatula* and included in it the species *serrata*. Humboldt (1811) established the genus *Porocephalus* to accommodate *P. crotali*. Rudolphi (1819) placed the species *taenioides, denticulatum, serratum, emarginatum* and *proboscideum* in a new genus which he named *Pentastomum*.

Within recent years the worms have been variously referred to as Linguatulids, Pentastomes and Porocephalids. It will be clear that the name Linguatula has priority, and this name is now used to designate the family.

Sambon (1922) defines the family LINGUATULIDAE as follows :—

'Blood sucking, endoparasitic *Acarina*. Body legless, elongate, vermiform, and more or less markedly annulated. Mouth round, squarish, or elliptical, provided with a chitinous armature of varied structure, and situated either before, behind or between two pairs of hollow, retractile, fang-like chitinous hooks emerging from four longitudinal pits or pouches disposed in trapezoidal formation or archwise. These hooks may be either equal or unequal, smooth or serrated, single or binate. Anus terminal or sub-terminal. Sexes divided ; female larger than male. Genital opening on mid-ventral line ; at anterior end of abdomen in male ; either at anterior or posterior end of abdomen in female. When at posterior extremity, the latter opens more or less near anus, but always anterior to it. The ovary is a long, dorsally-placed organ, usually extending almost the whole length of the abdomen. Anteriorly it divides into two oviducts, which bestride the alimentary canal, pass the spermathecae, receive the spermathecal ducts, and unite, forming either a wide egg-sac or a long tube, which combines the functions of both uterus and vagina. When tubular, the utero-vagina runs in a straight line to the posterior end in the virgin and permits copulation, but becomes greatly elongated in the gravid female, forming numerous and complex gyrations. Its coils may be amassed either above or below the alimentary canal, or they may be twined around it. Accordingly, the alimentary tube is either dorsal, ventral or axial. These anatomical details are given here because of their taxonomic importance. They are usually discernible in fully distended females owing to the transparency of the integument, but, in any case, they may be easily detected after clarification by the usual methods.

'The eggs are enclosed in a thin, bladder-like envelope filled with an albuminous substance clear as glass, and contain within their thick chitinous shell an oval embryo with rudimentary mouth parts and either six or four legs, each one tipped with two strong, claw-like hooks. On the back of the embryo may be seen the so-called dorsal organ or facette.'

Adult Linguatulids, although they occur occasionally in the gut or body cavity, are parasitic principally in the lungs of reptiles and in the frontal sinus and maxillary antra (connected with the nasal chambers) of the dog, the wolf and occasionally in man. They occur less rarely in birds and amphibians.

The larvae occur in animals which are preyed upon by the host in which the adult parasite lives.

In *Linguatula serrata* the eggs are passed in the discharge from the mouth and nostrils; they also occur in the faeces; each egg contains an embryo; when swallowed by the intermediate host the egg-shell is dissolved and the larva is liberated; the larva then bores its way to the liver, spleen, etc., and, after a series of moults it encysts, grows, and becomes a nymph. When this nymph is swallowed by the final host it makes its way from the gut to the lungs or the nasal cavities where it becomes adult; the nymph, when fully developed, is said to be able to leave the cyst and to migrate to the bronchi or to the intestine of the intermediate host, from which positions it is passed to the exterior. It reaches its final host by being sniffed up by the dog and becomes adult in the nasal cavities. It is also probable that, occasionally, the entire life-history is passed in one host.

Sambon (1922), in his Synopsis of the family LINGUATULIDAE, Parts I and II, classified the family as follows:—

Family LINGUATULIDAE, Shipley, 1898.

Sub-family 1. RAILLIETIELLINAE, Sambon, 1922.
Genera. *Raillietiella*, Sambon, 1910.
Reighardia, Ward, 1899.

Sub-family 2. POROCEPHALINAE, Sambon, 1922.

Section. *SEBEKINI*, Sambon, 1922.
Genera. *Sebekia*, Sambon, 1922.
Alofia, Giglioli, 1922.
Leiperia, Sambon, 1922.
Sambonia, Noc and Giglioli, 1922.
Diesingia, Sambon, 1922.

Section. *POROCEPHALINI*, Sambon, 1922.
Genera. *Porocephalus*, Humboldt, 1811.
Kiricephalus, Sambon, 1922.
Armillifer, Sambon, 1922.
Waddycephalus, Sambon, 1922.

Section. *LINGUATULINI*, Sambon, 1922.
Genera. *Linguatula*, Frölich, 1789.
Subtriquetra, Sambon, 1922.

KEY TO THE SUB-FAMILIES OF THE FAMILY LINGUATULIDAE.

Female genital opening anterior; mouth anterior to hooks. Salivary glands moderately developed; Larvae with six short legs..... *Raillietiellinae*

Female genital opening posterior; mouth in line with or posterior to hooks; salivary glands greatly developed. Larvae with four legs..... *Porocephalinae*

Sub-family I. RAILLIETIELLINAE, Sambon, 1922.

Diagnosis.—LINGUATULIDAE:

'Female genital opening at anterior end of abdomen. Utero-vagina straight, ample, sacciform. Mouth anterior to hooks. Salivary glands moderately developed. Larva with six, short, stumpy legs.'

Genus *Reighardia*, Ward, 1899.

Diagnosis.—RAILLIETIELLINAE:

'Body cylindrical, elongate, slightly attenuated at both ends. Integument covered with stud-like projections. Mouth in advance of hooks. Hooks exceedingly minute, placed in trapezoidal formation. Posterior extremity bluntly rounded and curved ventrally.

'Only one species known from Holarctic region. Adult form parasitic in air sac of Gulls and Terns (*Laridae*). Nymphal form probably encysted in fish.

'Type species: *Reighardia sternae* (Diesing, 1864), Ward, 1899.'

No specimens belonging to this genus were found in the collections of the Liverpool School of Tropical Medicine.

Genus *Raillietiella*, Sambon, 1910.

Diagnosis.—RAILLIETIELLINAE:

'Body cylindrical, long, slender, flattened ventrally, tapering at both ends. Integument smooth, transparent. Mouth well in advance of hooks; opens on a terminal projection. Hooks simple, unequal, placed in trapezoidal formation; anterior pair smaller than posterior. Three vesicular projections about each hook, one anterior, globular, ensheathing the hook, the other two hemispherical, one on each side. Two vesicular projections placed dorsally on each side of cephalothorax, on a level with anterior pair of hooks. Posterior extremity bifid; terminal lobes divergent. Anus between terminal lobes.

'Six species described from Oriental, Ethiopian and Neotropical regions. Adult form parasitic in *Ophidia*, *Lacertilia* and *Bufonidae*.

'Type species: *Raillietiella boulengeri*, Vaney and Sambon, 1910.'

KEY TO GENERA AND SPECIES OF THE SUB-FAMILY RAILLIETIELLINAE.

Hooks exceedingly minute without vesicular projections; posterior extremity bluntly rounded..... *Reighardia* with one species only, viz., *R. sternae*

Hooks each with three vesicular projections; posterior extremity bifid, terminal lobes divergent..... *Raillietiella*

KEY TO SPECIES OF THE GENUS RAILLIETIELLA.

1. Each hook bears one or two stylets on its inner surface, close to base..... *R. geckonis*
   Stylets absent.....2
2. Worms with over 38 annuli.....6
   Worms with less than 38 annuli.....3
3. Small worms under 8 mm. in length..... *R. indica*
   Worms over 8 mm. in length.....4
4. None of the hooks are borne on parapodia..... *R. boulengeri*
   Either one or both pairs of hooks are borne on parapodia.....5
5. Posterior pair of hooks borne on long parapodia..... *R. mabuiae*
   Both pairs of hooks borne on long parapodia..... *R. tetrapoda*
6. Body of female spirally coiled..... *R. spiralis*
   Body of female not spirally coiled..... *R. furcocerca*

As I have been unable to discover any morphological differences between the following species they are considered identical, viz.,

R. furcocerca.
R. furcocerca, var. *orientalis* and
R. furcocerca, var. *mediterranea.*

Raillietiella furcocerca (Diesing, 1835), Sambon, 1910.

SYNONYMS :—*Pentastoma furcocercum*, Diesing, 1835.
Pentastomum bifurcatum, Diesing, 1850.
Porocephalus bifurcatus, Shipley, 1898.

One damaged specimen of what appears to be this species, obtained from the pericardial region of a Colubrine snake in Southern Nigeria (February, 1915), was presented by Dr. J. F. Corson.

Raillietiella boulengeri, Vaney and Sambon, 1910.

SYNONYM :—*Porocephalus boulengeri*, Vaney and Sambon, 1910.

Three densely gravid females from *Causus rhombeatus*. Accra, January, 1915. Collected and presented by Dr. J. W. S. Macfie.

Sub-family II. POROCEPHALINAE, Sambon, 1922.

Diagnosis.—LINGUATULIDAE :

'Female genital opening at posterior end of abdomen. Utero-vagina tubular, greatly elongated and forming numerous windings. Mouth in a line with, or posterior to, hooks. Salivary glands greatly developed, extending whole length of body on either side of alimentary tube. Larva with four legs.'

KEY TO SECTIONS OF THE SUB-FAMILY POROCEPHALINAE.

	Body flattened	*Linguatulini*
	Body cylindrical.....1	
1.	Well-marked latero-ventral grooves; hooks in trapezoidal formation	*Sebekini*
	Latero-ventral grooves absent; hooks disposed archwise	*Porocephalini*

Section I. *Sebekini*, Sambon, 1922.

Diagnosis.—POROCEPHALINAE :

'Body cylindrical. Well-marked latero-ventral grooves. Hooks in trapezoidal formation. Alimentary canal dorsal, longer than body, sinuous.

'Type genus : *Sebekia*, Sambon, 1922.'

KEY TO GENERA OF THE SECTION SEBEKINI.

	Female genital pore on fifth ring from posterior extremity	*Sambonia*
	Female genital pore on terminal ring.....1	
1.	Worms more or less spirally coiled	*Leiperia*
	Worms not spirally coiled.....2	
2.	Convex surface of hooks serrated	*Sebekia*
	Convex surface of hooks not serrated.....3	
3.	Body capsicum-shaped. Annulations limited to ventral surface	*Diesingia*
	Body not capsicum-shaped. Annulations complete	*Alofia*

Genus *Alofia*, Giglioli, 1922.

SYNONYMS :—*Pentastomum*, Lohrmann, 1889.
Porocephalus, Shipley, 1898.
Reighardia, Sambon, 1910.

Diagnosis.—Sebekini :

'Body small, massive, banana-shaped. Cephalothorax large, continuous with abdomen. Annuli 70-75. Mouth **U**-shaped, very large, with posterior margin on posterior hook-line and anterior end above anterior hook-line. Hooks comparatively large, single, equal and smooth. Alimentary tube largely sinuous. Utero-vagina ventral, convoluted. Anus terminal. Genital opening contiguous.

'One valid and two doubtful species described. Two from Samoa, the third unknown locality. Hosts unknown, probably fish.

'Type species : *Alofia ginae*, Giglioli, 1922.'

Four species of this genus have been recorded, but at present it is not possible to prepare a key to the species as the details relating to them are so scanty. No species of the genus occurs in the

collection of the Liverpool School of Tropical Medicine. The principal details relating to the four species are as follows :—

TABLE I.

	Length	No. of annuli	Remarks
A. ginae ...	15 to 20 mm. × 2 to 3 mm.	75	Chitinous oral armature **U**-shaped.
A. merki ...	15 mm. × 3 mm.	75	
A. platycephala	23 mm. × 2.8 mm.	70	
A. adriatica ...	21 mm. to 85 mm.	72	Chitinous oral armature, key-shaped.

Genus *Sebekia*, Sambon, 1922.

SYNONYMS :—*Pentastoma*, Rud., 1819 (in part).
Pentastomum, Diesing, 1835.
Porocephalus, Stiles, 1893.
Reighardia, Sambon, 1910.

Diagnosis.—Sebekini :

'Body small, massive, closely annulated (annuli c. 80). Cephalothorax very small, wedge-shaped, projecting nipple-like from gross abdomen, ventral side continuous with that of abdomen. Mouth subterminal, shaped more or less like an inverted **U**, with free ends approximated. Hooks very small, distance between anterior and posterior hook-lines small. Hooks single, equal with convex surface serrated. Alimentary canal longer than body, forming sinuous loop about junction of anterior with medium third of body. Anus terminal. Utero-vagina long, much convoluted, amassed beneath alimentary tube. Ovary sinuous. Genital opening slightly anterior to anus.

'Six species described from Oriental, Ethiopian, Neotropical and Australasian regions. Adult forms parasitic in Crocodilians and Monitors. Nymphal forms probably in fish.

'Type species : *Sebekia wedli*, Giglioli, 1922.'

The details relating to the six species placed in this genus are so meagre that it is impossible to prepare a key. The principal point of differentiation between the species is said by Sambon to be the form of the chitinous oral armature surrounding the mouth. No species belonging to this genus were found in the collections of the Liverpool School of Tropical Medicine.

The following are the principal points relating to the six species described :—

Table II.

	Length	No. of annuli	Remarks
S. oxycephala ... ... ? = *P. crocodili*, Wheeler ...	10 mm.	60	
S. divestei	10 mm.	75	
S. wedli	10 to 25 mm.	80	
S. cesarisi	15 mm. × 2 mm.	Not known	Each annulus bears a pair of digitate processes.
S. indica	24 mm. × 5 mm.	Not known	
S. jubini	42 mm. × 4.5 mm.	Not known	

Genus *Sambonia*, Noc and Giglioli, 1922.

Diagnosis.—Sebekini :

' Body incurved, tapering at both ends. Annulations (44 in type sp.), slightly imbricative, giving the body outline a serrated appearance. Cephalothorax small, wedge-shaped. Mouth and hooks close to anterior border. Mouth ovate, placed between hook lines with longest diameter vertical. Hook-trapezoid, low and relatively wide at base. Hooks simple, smooth, equal. Anus terminal. Female sexual opening above terminal segment (fifth ring from posterior end in type sp.). Ova with strikingly characteristic spined outer shell. Parasite of Lizards.

'Type species : *Sambonia lohrmanni* (Sambon, 1910), Noc and Giglioli, 1922.'

Key to Species of the Genus Sambonia.

	Length	Annuli
S. lohrmanni	13 to 17 mm. × 3 mm.	44
S. wardi	75 to 150 mm.	44

No specimens of this genus were found in the collections of the Liverpool School of Tropical Medicine.

Genus *Leiperia*, Sambon, 1922.

Diagnosis.—Sebekini :

' Body large, cylindrical, elongate, more or less spirally coiled. Hooks in trapezoidal formation, simple, equal, smooth. Distance between hook-lines relatively great. Mouth small, situated on posterior hook-line. Utero-vagina beneath slightly sinuous alimentary tube. Posterior extremity tapering somewhat at the very end. Anus terminal. Female genital opening slightly anterior to anus.

' One species from Nilotic Crocodile (*Crocodilus niloticus*).

'Type species : *Leiperia cincinnalis* (Sambon, 1910), Sambon, 1922.'

The genus *Leiperia* contains one species only, viz., *L. cincinnalis*.

Leiperia cincinnalis, Sambon, 1922.

Very numerous young, adult, male and female specimens, all immature, from the lung of a crocodile. Upper Shire, Nyasaland. Collected by Professor R. Newstead, F.R.S., and Dr. Davey, in 1911.

The specimens measured from 35 mm. to 50 mm. in length and from 1 mm. to 1·3 mm. in breadth; the number of annuli varied from about 90 to 110. The female genital pore is situated on the last segment, slightly in front of the anus. The hooks are bifurcated, the larger branch measuring 212μ and the smaller 150μ in length. In the mature adult the hooks are simple.*

Genus *Diesingia*, Sambon, 1922.

Diagnosis.—Sebekini:

Body capsicum-shaped, the annulations being limited to the ventral surface; a median ventral groove is present.

Type species.—D. kachugensis (Shipley, 1910).

Sambon (1922) erected the genus *Diesingia* to accommodate *Porocephalus kachugensis*, Shipley, 1910, a capsicum-shaped nymph in which all the four hooks are bifid; it measured 12 mm. in length and 3 mm. in breadth, and had from 40 to 46 annuli; from *Kachuga lineata*, India.

Hett is of opinion that *D. kachugensis* is the larval form of *Subtriquetra megacephala* (Baird) and that, on account of its shape, etc., the latter species is intermediate between the genera *Linguatula* and *Subtriquetra*. If such proves to be the case then Baird's species will have to be referred to the genus *Diesingia*, and the latter genus to Sambon's section *Linguatulini*.

Section II. *Porocephalini*, Sambon, 1922.

Diagnosis.—Porocephalinae:

'Body cylindrical. No latero-ventral grooves. Hooks disposed archwise. Alimentary canal dorsal or axial, not longer than body, straight.

'Type genus: *Porocephalus*, Humboldt, 1811.'

* A number of smaller specimens of this species have since been obtained from 'a large fish,' Tanganyika.

KEY TO GENERA OF THE SECTION POROCEPHALINI.

The outer pair of hooks are bifurcated..... *Porocephalus*
Outer pair of hooks not bifurcated..... 1

1. Annulations marked by prominent bands giving the worm a beaded appearance..... *Armillatus*
Annulations not prominent.....2

2. Female pore opening considerably anterior to anus..... *Waddycephalus*
Female pore terminal..... *Kiricephalus*

Genus *Porocephalus*, Humboldt, 1811.

SYNONYMS :—*Echinorhynchus*, Humboldt, 1808.
Distoma, Humboldt, 1808.
Polystoma, Rudolphi, 1812.
Pentastoma, Rudolphi, 1819.
Linguatula, van Beneden, 1849.
Pentastomum, Diesing, 1850.

Diagnosis.—Porocephalini :

'Body club-shaped ; posterior half curved more or less ventrally ; terminal segments dilated into characteristic olive-shaped enlargement. Annuli smooth. Cephalothorax bluntly rounded anteriorly. Hooks unequal ; inner simple, outer provided with non-caducous accessory spine ; disposed in slightly arcuate line with convexity posterior. Mouth ovate, placed on inner hook-line. Anus subterminal. Female genital opening placed on terminal segment anterior to, and continuous with, anus. Alimentary canal axial, short, straight. Utero-vagina twined around alimentary tube. Four valid species described from Ethiopian and Neotropical regions. Adult forms parasitic in Ophidians ; nymphal forms encysted in mammals. Probably attacks man also.

'Type species : *Porocephalus crotali*, Humboldt, 1811.'

KEY TO SPECIES OF THE GENUS POROCEPHALUS.

Over 70 annuli..... *P. crotali*
Less than 70 annuli.....1

1. Annuli quite distinct.....2
Annuli indistinct and from 1 to 2 mm. apart..... *P. subulifer*

2. With 35 to 43 annuli..... *P. clavatus*
With 45 to 50 annuli..... *P. stilesi*

It should be noted here that the identity of *P. crotali*, Humboldt, is not definitely established. Hett (1924, pp. 141-144) discusses the question at some length. Humboldt obtained the species from a rattlesnake (*Crotalus cumanensis*) but did not state how many annuli the worm possessed. Diesing (1835 and 1850) described this and several other species under the name *Pentastomum proboscideum*, including one form from a boa and another only recorded hitherto from *Lachesis* spp. According to Diesing, the number of annuli is 80, but in his figures a varying number are shown. Leuckart examined Diesing's material

and stated that the worms possessed only about 40 annuli. Sambon accepts Diesing's statement that *P. crotali* has 80 annuli, but Hett (1924) agrees with Leuckart, and describes *P. crotali* as possessing about 40 annuli.

Porocephalus clavatus (Wyman, 1845), Sambon, 1910.

Synonyms :—Adult : *Linguatula clavata*, Wyman, 1847.
Linguatula proboscidae, van Ben., 1849, in part.
Pentastomum proboscideum, Leidy, 1856, in part.
Pentastomum clavatum, Leuckart, 1860.
Pentastomum imperatoris, Macalister, 1875.
Pentastoma moniliforme, Mégnin, in part.
Nymph : *Pentastomum didelphidis virginianae*, Leidy, 1852.

Specimens were obtained from the following sources :—(1) Two gravid females from a snake (genus and species unknown) : Leverville, Congo, October, 1923 ; collected by Dr. Brassart. (2) One specimen from a snake ; species and locality unknown. (3) Three specimens from a snake (*Causus rhombeatus*) : Gold Coast, September, 1915 : collected and presented by Dr. Corson.

This species has hitherto only been recorded from *Boa constrictor* and *Boa imperator*.

Most species of true boas inhabit the warmer parts of Central and Southern America, but two species occur in Madagascar.

Porocephalus subulifer (Leuckart, 1860), Stiles, 1893.

Synonym :—*P. cercopitheci*, Breinl and Hindle, 1908.

Several specimens from the lung of a Green Guenon (*Cercopithecus callitrichus*).

Genus *Kiricephalus*, Sambon, 1922.

Synonyms :—*Pentastomum*, Diesing, 1850.
Porocephalus, Shipley, 1898.

Diagnosis.—Porocephalini :

' Body club-shaped (like the Kaffir " kiri " and other knobbed clubs, sometimes armed with spikes), greatly elongate, of uniform thickness throughout and spirally twisted on its own axis. Annuli smooth. Cephalothorax more or less globular, owing to the constriction of anterior body rings brought about by habit of inserting cephalothorax deeply into host's lung. Hooks slightly unequal in size, but simple, without accessory spines. Mouth ovate, placed just below inner hook-line. Anus sub-terminal. Female sexual opening placed on terminal segment, somewhat anterior to anus. Alimentary canal axial ; on account of body torsion, appears to twine around utero-vaginal skein.

'Three valid species described from Oriental, Neotropical and Australasian regions. Parasitic in Ophidia.

'Type species : *Kiricephalus coarctatus* (Diesing, 1850), Sambon, 1910.'

KEY TO SPECIES OF THE GENUS KIRICEPHALUS.

	Length	Annuli
K. coarctatus	... 76 to 115 × 4 mm.	52
K. pattoni	... 80 to 115 × 2·5 mm.	36
K. tortus	... 40 mm.	25

Kiricephalus pattoni (Stephens, 1908), Sambon, 1922.

SYNONYM :—*Porocephalus pattoni*, Stephens, 1908.

The type specimens of this species from *Zamenis mucosus* are in the collections of the Liverpool School of Tropical Medicine. (1) Four females and one male were also obtained from a snake (species unknown) : Hong Kong ; presented by Dr. Bell. (2) Two females and one male from *Zamenis mucosus :* Zoological Gardens, Calcutta ; presented by Captain Knowles, I.M.S., 27.3.1923.

Genus *Armillifer*, Sambon, 1922.

SYNONYMS :—*Pentastoma*, Diesing, 1835.
Linguatula, Wyman, 1847.
Nematoideum, Diesing, 1851.
Pentastomum, Harley, 1856.
Porocephalus, Stiles, 1893.

Diagnosis.—Porocephalini :

' Body cylindrical, elongate, slightly curved ventrally ; terminal segment conical. Annulation strongly marked by thick, prominent bands in each segment, giving the species of this genus a beaded or ringed window-pole appearance. Cephalothorax wedge-shaped, with anterior border rounded. Hooks robust, equal, simple ; placed in straight or slightly arcuate line. Mouth orbicular, placed just above inner-hook line. Anus terminal. Female genital opening on terminal segment, somewhat anterior to anus. Alimentary tube dorsal, short, straight. Utero-vaginal coils amassed beneath alimentary tube. Three valid species described from Ethiopian and Oriental regions.

' Adult forms : Parasitic in Ophidia, nymphal forms in mammals and birds.

' Type species : *Armillifer armillatus* (Wyman, 1847), Sambon, 1922.'

KEY TO SPECIES OF THE GENUS ARMILLIFER.

With a neck-like constriction between cephalothorax and body.....3
Neck-like constriction absent.....1
1. With 28 to 35 annuli..... *A. moniliformis*
With less than 28 annuli.....2
2. With two small papillae in front of mouth..... *A. armillatus*
With a single small lobe in front of mouth..... *A. grandis*
3. Neck about 1 mm. in length..... *A. annulatus*
Neck 5 to 7 mm. in length..... *A. pomeroyi*

Sambon considers that *Porocephalus pomeroyi*, Woodland, is identical with *A. annulatus;* but Hett believes them to be distinct species and gives a table showing the differences between the two forms.

Armillifer moniliformis (Diesing, 1835).

SYNONYMS :—Adult : *Pentastoma moniliforme*, Diesing, 1835.
Pentastoma moniliforme, Leuckart, 1860.
Linguatule moniliforme, Mégnin, 1880.
Porocephalus moniliformis, Stiles, 1893.
Nymph : *Pentastomum tornatum*, Creplin, 1849, in part.
Pentastomum aonycis, Macalister ,1874.
Porocephalus armillatus, Stiles, 1908, in part.

Five female specimens (gravid) from *Tropidonotus picturatus*, Darwen, Northern Territory, Australia. Presented by Dr. P. A. Maplestone, D.S.O., M.B., Ch.B., Australia.

The adult form has hitherto only been recorded from the Indian python which inhabits India, Ceylon and Indo-China.

Armillifer armillatus (Wyman, 1847), Sambon, 1922.

SYNONYMS :—Adult : *Linguatula armillata*, Wyman, 1847.
Pentastomum polyzonum, Harley, 1856.
Pentastomum amillatum, Leuckart, 1860 (misprint).
Pentastoma armillata, Wyman, 1863.
Pentastomum armillatum, Diesing, 1864.
Porocephalus armillatus, Stiles, 1893.
Porocephalus polyzonus, Stiles, 1893.
Porocephalus moniliformis, Neumann, 1899 (in part).
Nymph : *Linguatula diesingii*, van Ben., 1849.
Pentastomum tornatum, Creplin, 1849 (in part).
Pentastomum euryzonum, Diesing, 1850.
Nematoideum hominis (*viscerum*), Diesing, 1851.
Pentastomum constrictum, Von Siebold, 1852.
Linguatula constricta, Küchenmeister, 1855.
Pentastoma leonis, Wedl, 1863.
Pentastomum leonis, Diesing, 1864.
Pentastoma tornatum, Cobbold, 1879.
Pentastomum protelis, Hoyle, 1883.
Porocephalus constrictus, Stiles, 1893.
Linguatula constrictor, Galli Valerio, 1896 (misprint).
Pentastomum diesingii, Shipley, 1898.

Specimens were examined from the following sources :— (1) Several nymphs from spleen, lungs, liver, etc., of man : Freetown, Gold Coast, West Africa ; collected by Dr. S. Adler, 18.12.1923. (2) Four specimens, nymphs, from liver of man ; presented by Dr. Manuk, Lokoja. (3) Two specimens from a dead prisoner ;

presented by Dr. J. Hannington, M.O., Nigeria. (4) Six specimens, immature adults, from a Kroo Boy : Degama, Gold Coast, July, 1913 ; Dr. H. A. Wilson ; collected and presented by Dr. J. W. S. Macfie. (5) Two nymphs from man, Akoada District, E.P. Nigeria, 13.9.13. ; collected by Dr. H. A. Wilson and presented by Dr. J. W. S. Macfie. (6) One nymph from man : Accra, Gold Coast ; collected by Dr. Findley, and presented by Dr. J. W. S. Macfie. (7) Several nymphs from liver of man, and one from mesentery : Accra, Gold Coast ; collected and presented by Dr. J. W. S. Macfie. (8) Several nymphs from man : Accra Gold Coast, October 15, 1916 ; collected and presented by Dr. J. W. S. Macfie. (9) Two specimens from the peritoneal cavity of a chimpanzee ; presented by Mr. Walker, 22.12.06. (10) One gravid female from a pig's liver : Accra, Gold Coast ; collected and presented by Dr. J. W. S. Macfie, 12.3.1923. (11) Three nymphs from liver of an ox : Accra, Gold Coast ; collected and presented by Dr. J. W. S. Macfie. (12) Several nymphs from liver of cattle : Accra, Gold Coast, 21.1.21 ; collected and presented by Dr. J. W. S. Macfie. (13) Very numerous adult specimens from a hedgehog : Accra, West Africa, 1.12.22 ; collected and presented by Dr. J. W. S. Macfie. (14) One adult female specimen measuring 80 mm. in length from lung of *Bitis masicornis*, Kumasi. (15) Three very large females from intestine of a horned *Cerastes*, Kumasi ; presented by Dr. Tweedy.

Genus *Waddycephalus*, Sambon, 1922.

Synonyms :—*Pentastoma*, Baird, 1862.
Pentastomum, Spencer, 1898.
Porocephalus, Shipley, 1898.

Diagnosis.—Porocephalini :

' Body club-shaped (like Australian " waddy "), tapering considerably towards posterior extremity, which ends in bilobed segment. Annuli smooth. Cephalothorax somewhat rounded, owing to constriction of anterior body segments. Hooks simple, unequal, inner larger, outer smaller, placed in arcuate line, the outer not only above the inner, but somewhat laterally. Mouth cordate, placed on inner hook-line. Anus between terminal lobes. Female genital opening considerably anterior to anus, placed on eighth body ring in *W. teretiusculus*. Alimentary canal axial, coils of utero-vagina amassed beneath alimentary canal.

' One species only described by Baird, in 1860, and again more fully by Spencer in 1893. Found in Australian snakes.'

KEY TO SPECIES OF THE GENUS WADDYCEPHALUS.

	Length	Annuli	Pore
W. teretiusculus	... 60 mm. × 5 mm.	Female 65 to 76 (50 to 70 Hett) Male 88 (60 Hett).	On 8th ring from anus
W. mazzai	?	41 to 44	On 3rd ring from anus.

Waddycephalus mazzai, Sambon, 1922.

SYNONYM :—*Pentastomum moniliforme*, Mazza, 1898.

Several adult female specimens from a snake, species unknown : Hong Kong ; presented by Dr. Bell.

Two species of this genus are known, viz., *W. teretiusculus*, found in the lungs of Australian snakes and *W. mazzai*, the host of which is unknown.

The specimens from Hong Kong agree in detail with the descriptions of *W. mazzai*.

Section III. *Linguatulini*, Sambon, 1922.

Diagnosis.—Porocephalinae :

'Body flattened fluke-like, more or less convex in middle part of dorsal surface, sides depressed. Hooks disposed archwise. Alimentary canal axial. Utero-vagina twines around it.

'Type genus : *Linguatula*, Frölich, 1789.'

KEY TO GENERA OF SECTION LINGUATULINI.

Body elliptical, flat ventrally, arched dorsally..... *Subtriquetra*
Body spatulate, attenuated posteriorly..... *Linguatula*

Genus *Subtriquetra*, Sambon, 1922.

SYNONYMS :—*Pentastoma*, Bresner, 1824.
Pentastomum, Diesing, 1850.
Linguatula, Railliet, 1883.

Diagnosis.—Linguatulini :

'Body more or less elliptical, flattened ventrally, greatly prominent dorsally. Adult form : Parasitic in crocodilians. Nymphal form : in fish.

'Type species : *Subtriquetra subtriquetra* (Diesing, 1835), Sambon, 1922.'

KEY TO SPECIES OF THE GENUS SUBTRIQUETRA.

Hooks simple..... *S. subtriquetra*
Hooks with subsidiary spines..... *S. megacephala*

The description of *Subtriquetra shipleyi*, Hett, 1924, is so meagre that it is impossible to distinguish it from *S. subtriquetra*.

Baird described the hooks of *S. megalocephala* as simple ; Sambon

states that they have accessory spines; but Hett, who examined the types, was unable to find accessory spines.

It has already been pointed out that it appears probable that *S. megacephala* is an intermediate form between the genera *Subtriquetra* and *Linguatula*. If Hett's opinion is correct, and it seems probable, then *S. megacephala* will have to be referred to the genus *Diesingia*, and the latter genus to the section *Linguatulini*, Sambon.

No specimens of this genus were found in the collections of the Liverpool School of Tropical Medicine.

Genus *Linguatula*, Frölich, 1789.

Synonyms :—*Taenia*, Pilger, 1803.
Halysis, Zeder, 1803.
Cochlus, Rudolphi, 1805.
Prionoderma, Rudolphi, 1808.
Polystoma, Rudolphi, 1809.
Echinorhynchus, Braun, 1809.
Tetragulus, Bosc., 1810.
Linguatula, Lamarck, 1816.
Pentastoma, Rudolphi, 1819.

Diagnosis.—Linguatulini :

'Body spatulate, attenuated posteriorly. Cephalothorax anteriorly obtuse. Mouth sub-terminal, squarish, situated between inner hooks. Hooks simple, equal, disposed archwise. Alimentary canal straight. Anus terminal. Uterus anteriorly twined round alimentary tube. Parasitic in mammals.

'Type species : *Linguatula serrata*, Frölich, 1789.'

Key to Species of the Genus Linguatula.

	Length	Posterior end
L. serrata	... 80 to 130 mm.	simple
L. recurvata	... 13 to 27 mm.	bifid and curved dorsally

Linguatula serrata, Frölich, 1789.

Synonyms :—Adult form.—*Ténia lancéolé*, Chabert, 1787.
Ver rhinaire, Chabert, 1787.
Taenia rhinaris, Pilger, 1803.
Taenia lanceolata, Rudolphi, 1805.
Cochlus rhinarius, Rudolphi, 1805.
Prionoderma rhinarium, Rudolphi, 1808.
Polystoma taenioides, Rudolphi, 1809.
Taenia rhinaria, Rudolphi, 1810.
Linguatula taenioides, Lamarck, 1816.
Prionoderma lanceolata, Cuvier 1817.
Pentastoma taenioides, Rudolphi, 1819.
Linguatula lanceolata, de Blainville, 1828.
Linguatula rhinaria, Railliet, 1900.

Nymph : *Linguatula serrata*, Frölich, 1789.
Taenia capraea, Abildgaard, 1789.
Taenia caprina, Gmelin, 1800.
Polystoma serratum, Goeze, 1803.
Halysis caprina, Zeder, 1803.
Linguatula denticulata, Rudolphi, 1805.
Echinorhynchus caprae, Braun, 1809.
Polystoma denticulatum, Rudolphi, 1809.
Tetragulus caviae, Bosc., 1810.
Pentastoma denticulatum, Rudolphi, 1819.
Pentastoma serratum, Rudolphi, 1819.
Pentastoma emarginatum, Rudolphi, 1819.
Pentastoma fera, Creplin, 1829.
Pentastoma taenoides, Dick, 1840 (misprint).
Linguatula ferox, Gros, 1849.
Linguatula caprina, R. Blanchard, 1895.
Linguatula rhinaris, Railliet, 1900.

(1) One specimen from a dog's nose; presented by Captain Carter, I.M.S., India. (2) A number of nymphs from the liver of a male bushbuck (*Tragelaphus scriptus*) : Upper Shire, Nyasaland; collected and presented by Professor R. Newstead, F.R.S., and Dr. Davey; 1911. (3) One specimen from the nose of a dog: Manchester, England; lent by A. W. Noel Pillers, Esq., F.R.C.V.S., D.V.S.M. Mr. Pillers states that he has only obtained this parasite twice in twenty years in England, once as noted above, and a second specimen from the nasal cavity of an otter hound in Shropshire.

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NOTES ON SOME AFRICAN CERATOPOGONINAE—SPECIES OF THE GENUS *FORCIPOMYIA*

BY

A. INGRAM

AND

J. W. S. MACFIE

(*Received for publication* 13 *October*, 1924)

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The collections of Ceratopogonine midges made by us at Accra, mostly between the years 1919 and 1922, contained very numerous specimens of *Forcipomyia ;* indeed, certain species of this genus (*e.g.*, *F. castanea*, *F. ingrami*, and a species allied to *F. chrysolopha*) were amongst the most abundant in the laboratory where the majority of our specimens were obtained. The task of examining so large a number of specimens has been no light one and for unavoidable reasons has only been completed recently, the results being embodied in the following paper. In addition to our Accra collections we have had at our disposal a small number of specimens from other places in the Gold Coast and from Nigeria, and we have been

privileged, thanks to the kindness of Dr. G. A. K. Marshall, to examine a small collection of *Forcipomyia* belonging to the Imperial Bureau of Entomology. This collection, which is largely composed of specimens taken by ourselves before we began our investigation, contains specimens from various parts of East and West Africa, and although not including any species not previously identified in our own collections, extends in several cases the range of distribution.

The large number of specimens at our disposal has enabled us to study in a few species the important question of the range of variation, with results which are interesting although rather disconcerting. We have found, for example, that such a character as the presence of bands on the legs is liable to great variation, and that colour markings are sometimes quite unreliable as specific characters. In a long series of specimens of *F. ingrami* we have found the general colouring to vary from very dark brown to quite a pale, yellowish-brown ; in *F. castanea* the characteristic sepia-brown band on the hind femora may vary considerably in size and depth of colour, may be reduced to a mere spot, or may be entirely absent ; and in *F. inornatipennis* the legs may be uniformly coloured, or may show dark bands on the hind femora only, on the hind femora and tibiae, or on both middle and hind legs. On the other hand, identical colour markings may be no sure indication of specific identity, for from a long series of specimens somewhat resembling *F. chrysolopha*, we have separated males of three species with quite dissimilar genitalia which we are unable to distinguish by any other characters. Such observations inevitably shake confidence in specific differentiations based mainly or entirely on colour markings, and make it difficult or impossible to estimate the value of descriptions, written perhaps many years ago, at a time when morphological characters, especially those of the genitalia, were either ignored or referred to but briefly. In the present state of knowledge it appears impossible to tell whether certain species of *Forcipomyia* are really different or differ only in description, and no decision can be reached without a re-examination of the type specimens.

In the descriptions of species which follow, we have given a somewhat detailed account of *F. castanea*, because we have in our possession abundant adult specimens of this species, both males and

females, and also pupae, larvae, and eggs, and have curtailed so far as possible the descriptions of the other species. The unit frequently referred to in the text is 3·7μ. All measurements were made from specimens immersed in pure carbolic acid, and thus restored to their natural size and not shrunken as they tend to be when dry. The midges vary considerably in size, the ratios of the measurements are therefore probably of greater value than the actual measurements themselves.

The types and co-types of the new species described have been deposited in the collection of the Liverpool School of Tropical Medicine.

In some of our previous papers we have given keys for the identification of the Gold Coast species of certain genera of Ceratopogoninae. In this paper we venture to go a step further and have attempted a key for the identification of the African species of the genus *Forcipomyia*. We have done this, because it is now known that many Ceratopogonine midges are widely distributed in Africa (*e.g. Culicoides schultzei* in East, South, and West Africa, and in Egypt) and it is probable that, as time goes on, many more will be found to extend over a large part of the Continent, including regions with dissimilar climates. Some species, indeed, appear to have an even more extensive range of distribution, and although it is certainly artificial to make a limit of the shores of the Mediterranean, the Suez Canal, and the Red Sea, it appears to us that no grouping would be entirely satisfactory which did not include all known species (a project at present impracticable), but that in the meantime a key to the African species would be useful.

In drawing up our key we have used primarily the following characters in order :—the presence or absence of scales, the relative lengths of the first and second tarsal segments of the hind legs, the presence or absence of pale coloured spots on the wings, the adornment of the legs, and the form of the antenna of the females. They are all characters readily seen with a hand-lens or low-power microscopic objective, excepting the first, which we felt compelled to use as it has been made a generic character by Kieffer and certainly appears to separate off a somewhat compact group of species, which we regret is not always easy to make out in dry specimens. We have encountered great difficulties, to some of which we have already

referred. Unfortunately, also, only one sex is known of many species; the genitalia of males (which furnish most valuable differential characters) are often described imperfectly, as seen with a hand lens; there is often doubt as to whether descriptions are adequate in the case of important characters (such as the presence of scales) not easily determined at low magnifications; and with the exception of the species described below and two others from Egypt previously described by one of us, we have had no opportunity of augmenting the published descriptions by reference to the insects themselves. Our key, therefore, can only be regarded as tentative. It almost certainly includes as different species several which are actually identical.

GENUS *FORCIPOMYIA* (Megerlé, 1818) Kieffer, 1901

External Morphology.

Adults. Cuticle all over the body covered by minute upright microtrichia. *Head.* Eyes usually bare; but the cuticle between the eyes and the bases of the antennae is covered by minute hairs, and a very few similar hairs may be found occasionally between the facets at the margin of the eyes; no account is taken, however, of such hairs in the descriptions of species which follow. Eyes usually broadly contiguous above in both sexes, the facets separated by a narrow line. Vertex and occiput, in both sexes, bearing numerous, stout, forwardly-projecting hairs, and sometimes scales also. Immediately in front of the anterior border of the eyes is a conical thickening of the cuticle or callosity which is sometimes prominent. Clypeus pronounced, especially in females, hairy.

Mouth-parts. Proboscis rather longer than the head in females, shorter in males. The component organs generally similar to those of *Culicoides* (see *F. castanea*), mandibles armed with numerous small teeth, maxillae with fewer, delicate, widely-spaced teeth. Palpi in both sexes composed of five segments, bearing moderately long and short hairs. Second, fourth, and fifth segments usually about sub-equal, about twice as long as broad. The third segment the largest, usually as long as the fourth and fifth together, inflated about the middle or basally, in some species very greatly, and containing a sub-spherical or oval sensory pit opening on the inner

aspect, in which are a number of minute, modified, drumstick-like, sensory hairs. In some species modified sensory hairs are present also on the surface of the third segment on its inner aspect. The fifth segment only slightly if at all expanded at its end. In the males the palpi are usually more slender than in the females, and the third segment inflated to a lesser degree and furnished with a smaller sensory pit.

Antennae. Composed of fifteen segments, not sculptured ; pilose in the female, plumose in the male. In the female the first segment small, bearing stout hairs which are directed anteriorly and are sometimes pubescent. Torus sub-spherical, bearing a few small hairs. Third segment sub-spherical or oval, larger than the fourth, with a posterior stalk of variable length. Segments four to ten usually sub-spherical to oval, sometimes flask-shaped, becoming progressively longer and narrower towards the tenth ; segments eleven to fifteen sometimes forming an almost continuous series with the basal segments, sometimes elongated, sub-cylindrical, an abrupt change of shape taking place between the tenth and eleventh segments. The combined length of segments 3 to 10 usually greater than that of segments 11 to 15. The last segment is the longest and ends in a blunt, often club-shaped, stylet. Hairs on the basal segments short, about twice the length of the segments or less, arranged in whorls of frequently about a dozen or more hairs. Hairs on the five distal segments usually rather shorter, those arising posteriorly the longest, forming a whorl, the rest scattered. All the flagellum segments bear small, colourless spines ; the basal segments bear also longer spines which are usually curved or geniculate, and may be very stout and either pointed or blunt. In the male the first segment devoid of hairs. Torus very large, shaped like a Dutch cheese hollowed out in the middle anteriorly, without hairs. Third segment sub-spherical, smaller than the fourth, from which it is separated by a wide membranous interspace, with a rather long stalk. Segments four to eleven from sub-spherical to irregularly ovate, broadly united, gradually becoming narrower and more drawn out anteriorly towards the eleventh ; segments twelve to fifteen elongated, sub-cylindrical, sharply separated from one another, the twelfth usually much the longest and shaped like the eleventh, but with the distal end greatly extended, the relative lengths of the other three varying

in different species. The last segment is broader than the others and ends in a blunt, often club-shaped stylet. Large spines are present on the basal, and smaller spines on all the segments of the flagellum ; and on segments four to twelve are large whorls of hairs, transverse on the basal segments but oblique on the more distal ones, forming a plume which reaches nearly to the level of the end of the antenna. The whorl on segment three is reduced ; on segments thirteen to fifteen are numerous shorter hairs, those at the bases of segments thirteen and fourteen arranged as small whorls.

Thorax strongly arched, but not projecting anteriorly over the head. Thoracic pits absent. Dorsum clothed with short hairs and sometimes scales, and bearing laterally and posteriorly a few longer, stouter hairs, some of which in some species form a curved row on each side a little anterior to the scutellum. Dorsum often adorned with three broad longitudinal stripes separated by narrow, paler-coloured lines, the middle stripe deficient posteriorly, and the lateral ones deficient anteriorly ; more laterally there is often an additional, small, narrow, longitudinal stripe on each side. Scutellum a transverse strip of chitin with a concave anterior and convex posterior border ; bearing numerous bristles and hairs and sometimes scales. Post-scutellum devoid of hairs.

Wings usually unadorned, but sometimes with one or more pale spots. Surface of the wings entirely covered with minute microtrichia which vary in size in different species to some extent, but are smaller than those of *Atrichopogon ;* and more or less densely clothed with larger, decumbent hairs or scales. The decumbent hairs are more or less lanceolate and curved, less straight and rigid-looking than in *Atrichopogon* and not tapering so regularly to sharp points; they are sometimes sub-plumose. The hairs are usually denser and darker on the anterior part of the wing, but there is a small area in this region, immediately distal to the end of the costa, which is bare. There are no bare areas along the veins as in *Atrichopogon.* The fringe on the posterior border of the wing is long, composed of one or two rows of long hairs between two rows of shorter, oblique hairs—the hairs lanceolate, angled or curved, and sometimes pubescent or even sub-plumose. The wing of the female is broad, in most species broadest near the base, with a rounded tip and a well-formed anal

angle ; that of the male is longer and narrower, and usually much paler in colour. The costa reaches to about the middle of the wing. The first and third veins are more or less fused basally, the first radial cell being completely absent or reduced to a mere slit, visible only with the aid of staining reagents. Distal radial cell well-formed, generally triangular, but varying in size and shape in different species. Radio-medial cross-vein oblique. The distance from the cross-vein to the base of the fifth vein greater than the distance from the cross-vein to the end of the costa. Fourth vein with a short petiole, about the same length as the cross-vein ; rami long, more or less obsolete proximally. Fifth vein forking near the level of the end of the costa, either a little distal to it or a little proximal to it ; anterior ramus continuing the line of the stem ; in most species both rami reaching the margin of the wing at a level distal to that of the end of the costa. Intercalary veins well-developed ; that between the end of the costa and the tip of the wing Y-shaped.

Legs often adorned with darker bands or spots on the femora and tibiae, and tarsal segments frequently infuscated ; somewhat densely clothed with hairs which are sometimes very long, and in certain species bearing also scales. Femora not greatly swollen, unarmed, but occasionally bearing hastate spines. Tibiae not greatly swollen ; occasionally bearing hastate spines. Fore tibiae armed at the distal extremity with a stout spur, a patch of strong, spine-like hairs, and an oblique transverse row of short, fine bristles ; middle tibiae usually unarmed, but a few bristles in a transverse row near the apex are highly developed and may be of great length ; hind tibiae with a stout spur and two transverse rows of graded bristles apically which are similar to those of *Culicoides*, but composed of more numerous elements. Tarsal segments with apical bristles on the first four segments differentiated, spine-like ; and a more or less complete longitudinal row, or rows, of similar differentiated bristles on the three first segments. Fifth tarsal segment unarmed. . All the tarsal segments cylindrical ; the first may be longer or shorter than the second or of equal length, the third, fourth, and fifth progressively diminishing in length. Claws equal, about half the length of the last tarsal segment, sharply curved ; in the female simple, in the male usually with bifid ends, and more delicate and more sharply curved than in the female.

Empodium about as long as the claws, with long hairs on both lateral margins.

Abdomen short and broad in the female, longer and more slender in the male ; not petiolate. Hypopygium of the male large and conspicuous. Lamellae of the female small and rounded. Well-clothed with hairs and sometimes scales. Spermathecae usually two in number, highly chitinised, oval or pyriform in most species ; the commencement of the duct often chitinised for a short distance.

External genitalia of the male. The hypopygium is relatively large and complex, and presents morphological characters which are of value in distinguishing closely-allied species, and in some cases indeed have been the only structures in which we have been able to detect specific differences. In describing the various structures composing the hypopygium we have adhered to the nomenclature used in our previous papers.

The *ninth segment* is well-chitinised, often somewhat narrowed anteriorly, so that the tergite which is narrowed posteriorly also, has a shape suggestive of the ' lozenge ' ; both tergite and sternite usually well-clothed with moderately long hairs. Sternite usually not notched in the middle line posteriorly. Tergite relatively short, prolonged posteriorly as a membranous extension bearing dorsally a pair of long, stout hairs, and terminating laterally in small, irregularly-shaped processes which are often slightly chitinised and bear usually two long and two short hairs. *Forceps* well-developed, highly chitinised. Side-pieces large, oval, densely clothed with hairs, some of which are often of great length. Dorsal root-like process long and slender, highly chitinised, often appearing to be jointed at its base. Claspers about as long as the side-pieces, usually less highly chitinised, broad at the base and narrowing rapidly towards the apex ; clothed at the base at least with minute hairs intermixed with which are a few larger hairs, and bearing at the apex a few minute hairs. *Harpes* usually in the form of two chitinised admedian plates or rods which project posteriorly and slightly ventrally, may or may not be joined anteriorly across the middle line by a strip of chitin, and articulate by their proximal ends with the distal ends of the dorsal root-like processes at the bases of the side-pieces. In some species, however, no such posteriorly projecting structures are present, and the dorsal root-like

processes at the bases of the side-pieces join across the middle line anteriorly. The harpes vary in shape and size in different species, and are of great systematic value. *Aedoeagus* a complicated structure, more or less conical, triangular, shield-shaped, or stirrup-shaped in ventral-view, and largely membranous or but feebly chitinised. The aedoeagus varies greatly in shape in different species and is of value in distinguishing them.

PUPA AND LARVA. A detailed morphological study of the pupa and larva of *Forcipomyia* has recently been published by Saunders. As we succeeded in obtaining materials illustrating the early stages of only two species, and as Saunders states that he expects shortly to publish descriptions of the early stages of further, exotic species, it is only necessary for us to refer here to his work.

KEY TO THE AFRICAN SPECIES OF THE GENUS *FORCIPOMYIA*

1. With scales (*Lepidohelea*) 2
   Without scales 15

2. First tarsal segment of hind legs longer than second*..... 3
   First tarsal segment of hind legs shorter than second or of about equal length..... 4

3. Legs entirely brown ; first tarsal segment of hind legs only slightly longer than second ..... *F. armaticrus*, K.†
   Femora and tibiae dark brown, tarsi yellowish ; first tarsal segment of hind legs decidedly longer than second ..... *F. auripes*, sp.n.

4. Wings adorned‡..... 5
   Wings unadorned 9

5. Wings with a single pale spot, situated about the middle of the anterior border, in the region of the end of the costa 6
   Wings with several pale spots 7

* In all the species examined by us [excepting *F. ingrami*] the ratio of the length of the first tarsal segment of the hind legs to the second, although varying somewhat, is similar in the two sexes, and in this key where only one sex is known we have assumed that the ratio is approximately the same in both sexes.

† It is dificult to place this species in the key correctly, because part of the description appears to have been dropped out by the printer ; we have, therefore, inserted it twice.

‡ By adornment we mean with pale spots. No account is taken, however, of the usual small bare area just beyond the end of the costa which may simulate a spot, nor of appearances due to the darkening of the anterior part of the wing produced by greater density of hairs and scales in this region, nor of the pallor sometimes visible at the extreme base of the wing.

6. Hind femora with a dark brown apical band, and hind tibiae with a dark brown basal band ..... *F. biannulata*, sp.n.
Hind femora with a narrow sepia-brown band or spot a little distal to the middle ..... *F. castanea*, (Walk.)
Hind femora without bands or spots..... *F. castanea* (Walk.) var. *incomptifeminibus* (Aust.)

7. Antenna of female with last segment twice as long as broad ; that of the male with 13th and 14th segments sub-equal, about half the length of the 12th..... *F. chrysolopha* (K.)
Antenna of female with last segment about three or four times as long as broad ; that of the male with 13th segment longer than the 14th and more than half the length of the 12th 8

8. Harpes of male large, hook-like ; antenna of female with segments 4 to 10 flask-shaped, about two to two-and-a-half times as long as broad, bearing long, slender, pointed spines ..... *F. squamipennis*, sp.n.
Harpes of male long, stout, with rather blunt ends ; antenna of female with segments 4 to 10 less than twice as long as broad, bearing very stout, blunt spines ..... *F. lepidota*, sp.n.
Harpes of male long, with filiform ends ; antenna of female as in *F. lepidota* ..... *F. venusta*, sp.n.
Harpes of male long, with ends a little expanded like a duck's head ; female unknown..... *F. pampoikila*, sp.n.

9. Hind femora with dark bands or other markings 10
Hind femora without dark bands or other markings 13

10. Hind tibiae almost entirely dark brown, only the extreme apex being slightly tawny ..... *F. nigrotibialis*, sp.n.
Hind tibiae with at most a single dark brown band... *F. inornatipennis* (Aust.) var. *ornaticrus*, var.n.
Hind tibiae with two dark brown bands 11

11. Bands on the hind tibiae at the extremities, with a clear ring between them ..... *F. nilotica* (K.)
Distal dark band on the hind tibiae not reaching the extremity of the segment 12

12. Darkish brown ; very hairy ; antenna of male with the combined length of segments 3 to 11 almost equal to that of segments 12 to 15, segment 12 three-and-a-half times as long as segment 11 ... *F. hirsuta*, sp.n.
Yellow or whitish ; antenna of male with combined length of segments 3 to 11 as long as that of segments 12 to 15, segment 12 two-and-a-half times as long as segment 11..... *F. ornatipes* (K.)
Light brown ; antenna of male with combined length of segments 3 to 11 greater than that of 12 to 15, segment 12 only twice the length of segment 11..... *F. tigripes*, sp.n.

13. First tarsal segment of hind legs slightly longer than second..... *F. armaticrus*, K.
First tarsal segment of hind legs clearly shorter than second, one-half to two-thirds the length 14

14. Legs yellowish; terminal segments (11 to 15) of antenna of female over four times as long as broad; spermathecae oval, 80-130 μ by 60-80 μ, practically no part of the duct chitinised ..... *F. inornatipennis* (Aust.)
Legs brown; terminal segments of antenna of female about three times as long as broad; spermathecae smaller, subspherical, diameter about 40 μ, the commencement of the duct chitinised for a short distance, about 7 μ ..... *F. nilotheres*, M.

15. First tarsal segment of hind legs longer than the second.....16
First tarsal segment of hind legs shorter than the second, or of about equal length.....30

16. Wings adorned 17
Wings unadorned 18

17. Wings with two pale, yellowish, elongated spots on the anterior border, one covering the distal end of the third vein, and one between the anterior border of the wing and the fork of the intercalary vein; first and third veins forming a small distal cell ..... *F. tangae*, K.
Wings with grey markings and two dark spots on the anterior border; first and third veins completely fused ..... *F. puncticollis* (Becker)

18. Eyes hairy ..... *F. aethiopiae*, sp.n.
Eyes bare.....19

19. Antenna of female with the last five segments elongated, an abrupt change of shape between the tenth and eleventh segments 20
Antenna of female with flagellum segments forming an almost continuous series, no abrupt change of shape between the tenth and eleventh segments 25

20. First tarsal segment of hind legs only slightly longer than the second, less than twice as long.....21
First tarsal segment of hind legs twice the length of the second or longer.....23

21. First and third veins of the wing joined for their whole length ..... *F. aplanota* (K.)
First and third veins forming a distinct distal cell 22

22. Reddish, with pale yellow legs (*i.e.*, as in *F. aplanota*); first and third veins of wing fused in proximal half; scutellum brown (*i.e.*, as in *F. aplanota* female) ..... *F. seychelleana* (K.)
As *F. seychelleana* but thorax orange-red and abdomen very dark brown..... *F. seychelleana* var. *fulvithorax* (K.)

Reddish, with pale yellow legs; first and third veins of the wings fused in proximal two-thirds; scutellum yellow in female, dark brown in male... *F. rufescens* (K.)

Usually dark brown, but sometimes paler brown, with yellowish-brown legs; first and third veins of wing in female with proximal halves contiguous, but actually forming a very narrow proximal cell difficult to distinguish; scutellum brown in both sexes ..... *F. ingrami*, ♀, Carter *

Yellowish-white or reddish, with yellow legs; first and third veins of wing confluent in their proximal halves; scutellum (female) brown or dark brown ..... *F. kribiensis* (K.)

23. Terminal segments of antenna of female three to four times as long as broad; very dark brown species with yellowish legs; bifurcation of the fifth vein of wing proximal to the end of the costa..... *F. mahensis* (K.)

Terminal segments of antenna of female twice as long as broad 24

24. Very dark brown, legs brownish ..... *F. falcinella* (K.)

Yellow, legs whitish ..... *F. sulfurea*, K.

25. First tarsal segment of hind legs about one-and-a-half times as long as the second 26

First tarsal segment of hind legs over twice the length of the second 28

26. Dorsum of thorax with three broad, brown, longitudinal stripes ..... *F. abyssiniae* (K.)

Dorsum of thorax uniformly coloured 27

27. First and third veins of the wing united ..... *F. tavetae*, K.

First and third veins of the wing forming a distal cell ..... *F. egypti*, M.

28. Dorsum of thorax with three broad, darkish brown, longitudinal stripes ..... *F. exigua*, sp.n.

Dorsum of thorax uniformly coloured 29

29. Antenna of female with combined length of segments 3 to 10 one-quarter longer than that of segments 11 to 15; whorls of 6 or 7 hairs ..... *F. seneveti*, K.

Antenna of female with combined length of segments 3 to 10 equal to that of segments 11 to 15; whorls of 10 to 12 hairs ..... *F. seneveti* var. *biskraensis*, K.

30. Wings adorned 31

Wings unadorned 36

31. Wings with several pale spots ..... *F. pretoriana* (K.)

Wings with a single pale spot about the middle of the anterior border, in the region of the end of the costa 32

* In the male *F. ingrami*, the first tarsal segment of the hind legs is shorter than the second.

32. Legs with darker brown bands or spots 33
Legs without such markings 34

33. Hind femora with a narrow sepia-brown band or spot a little distal to the middle, hind tibiae unadorned; harpes of the male with pointed but not filiform ends..... *F. castanea* (Walk.)*
Hind femora with a dark brown apical band, hind tibiae with a dark brown basal band; harpes of male with filiform ends, but no patch of teeth at the base of the side-pieces..... *F. biannulata*, sp.n.*
Hind femora with an inconspicuous spot at the distal end, hind tibiae sometimes with a trace of a band a little below the knee; harpes of male with filiform ends, side-pieces with a patch of spines or teeth on the inner basal aspect ..... *F. ashantii*, sp.n.

34. Whitish; combined length of segments 3 to 10 of antenna of female a half greater than that of segments 11 to 15 ..... *F. leucochaeta* (K.)
Brown or dark brown; combined length of segments 3 to 10 of antenna of female only slightly greater than that of segments 11 to 15...35

35. Last five segments of the antenna of the female elongated, segments 11 to 14 two to three times as long as broad; an abrupt change of shape between segments 10 and 11 ..... *F. castanea* (Walk.) var. *incomptifeminibus* (Aust.)*
Last five segments of antenna of female not so much elongated, segments 11 to 14 nearly twice as long as broad; change of shape between segments 10 and 11 not abrupt..... *F. striaticornis* (K.)

36. Legs with darker brown bands or spots.....37
Legs unadorned with darker markings 41

37. Hind legs with a dark spot at the distal end of the femora only.....38
Hind legs with bands on both femora and tibiae.....39

38. Thorax dark brown; terminal segments of the antenna of the female three times as long as broad ..... *F. lasionota* (K.)
Thorax pale yellow; terminal segments of the antenna of the female only twice as long as broad ..... *F. lasionota* var. *callithorax* (K.)

39. Yellow; thorax with three broad, brown, longitudinal bands on the scutum..... *F. fusciforceps* (K.)
Mainly dark brown, with yellow legs; thorax without dark longitudinal bands on the scutum 40

* *F. castanea*, *F. castanea* var. *incomptifeminibus* and *F. biannulata* are inserted in the key twice as the scales are apt to be overlooked.

40. Hind legs with distal third of femora and proximal third of tibiae brown ..... *F. kilemae*, K.
Hind legs with femora except the base, and tibiae except the extremity, brown ..... *F. sabariensis*, K.

41. Legs dark brown 42
Legs yellowish 43

42. Very dark brown; dorsum of thorax showing indistinctly three broad longitudinal stripes ..... *F. melanchroa*, sp.n.
Dull darkish brown; dorsum of thorax uniformly darkish brown ..... *F. nigeriensis*, sp.n.

43. Females known 44
Only males known 45

44. Terminal segments of the antenna of the female elongated, clearly differentiated from the basal segments..... *F. maura* (K.)
Terminal segments of the antenna of the female not clearly differentiated from the basal segments; no abrupt change of shape between segments 10 and 11..... *F. radiifer* (K.)

45. First and third veins of the wing confluent, no cell ..... *F. niligena* (K.)
First and third veins forming a distal radial cell...46

46. Very dark brown, legs pale yellow; hind tarsi with the first segment less than half the length of the second..... *F. psilonota* (K.)
Not so dark brown; hind tarsi with the first segment only slightly shorter than the second 47

47. Harpes without rod-like posterior structures ..... *F. ingrami*, ♂, Carter
Harpes with rod-like posterior processes which terminate in sharp points ..... *F. catanei*, K.

FORCIPOMYIA CASTANEA (Walk.)

Length of body* (average), 2 mm.; length of wing, 1·4 mm.; greatest breadth of wing, 0·5 mm. The size is variable, the length of the body of the females measured by us ranging from 1·4 mm. to 2·2 mm. In the male the length of the body is greater than in the female, owing to the size of the hypopygium, and the wings are narrower.

Head dark sepia-brown; occiput bearing numerous, long, dark brown hairs which overhang the eyes. Eyes bare†; broadly

* In all cases this measurement is taken from the anterior margin of the thorax to the tip of the abdomen of specimens mounted in pure carbolic acid.

† The cuticle between the eyes and the bases of the antennae is covered with minute hairs, and a very few similar hairs may be found occasionally between the facets at the extreme inner margins of the eyes. No account is taken of such hairs, however, in the description of this insect, or of the following species.

contiguous above in both sexes, but with the facets separated by a narrow line. Clypeus dark brown ; in the female somewhat elongated, bluntly rounded distally, bearing about sixteen dark brown hairs which are mostly grouped along the middle line ; in the male shorter, more rounded. Proboscis dark brown, in the female rather longer than the head, in the male shorter. Labium shorter and broader than in *Culicoides*. Stylets well-developed, chitinous. Labrum in the female highly chitinised, with sides parallel to the distal fourth, then gradually tapering to a narrowly rounded apex and bearing a delicate fringe ; in the male similar but less highly chitinised and more sharply attenuated distally, with dense hair-like processes over the distal third. Mandibles in the female long, broad, strongly chitinised, blade-like and pointed, with numerous (about twenty-five) closely apposed, blunt teeth extending almost half the length of the inner margin ; in the male similarly shaped, well-developed, highly chitinised but not so strongly as in the female, without teeth. Maxillae very similar to those of *Culicoides*, extending forwards rather less than the mandibles, less highly chitinised, more delicate and narrower, the distal fourth with about ten delicate, rather widely spaced teeth ; in the male well-developed, thinly chitinised, apex with fairly long and strong hairs. Hypopharynx in the female highly chitinised, similarly shaped to that of *Culicoides*, but distally somewhat narrower, broad at the base, tapering gradually to the distal fourth and then more rapidly to the pointed apex, distal margin smooth ; in the male as in the female, dense, sharply pointed. Palpi (fig. 1, A and B) dark brown, bearing rather numerous and long dark brown hairs : first segment small, second, fourth, and fifth small, sub-equal, third large, longer than any two of the other segments together, basal half swollen and furnished with a large, oval sensory pit with a circular orifice. *Antennae* uniformly darkish brown, bearing similarly coloured hairs. In the female segments 3 to 10 bearing long, stout spines which are almost straight, colourless, and about as long as the segments, and whorls of about twelve dark brown hairs which are about twice as long as the segments ; segments 11 to 15 bearing numerous smaller spines and small basal whorls of hairs. First segment a ring of dark brown chitin, bearing numerous dark brown hairs. Torus dark brown, sub-spherical, bearing a few dark brown

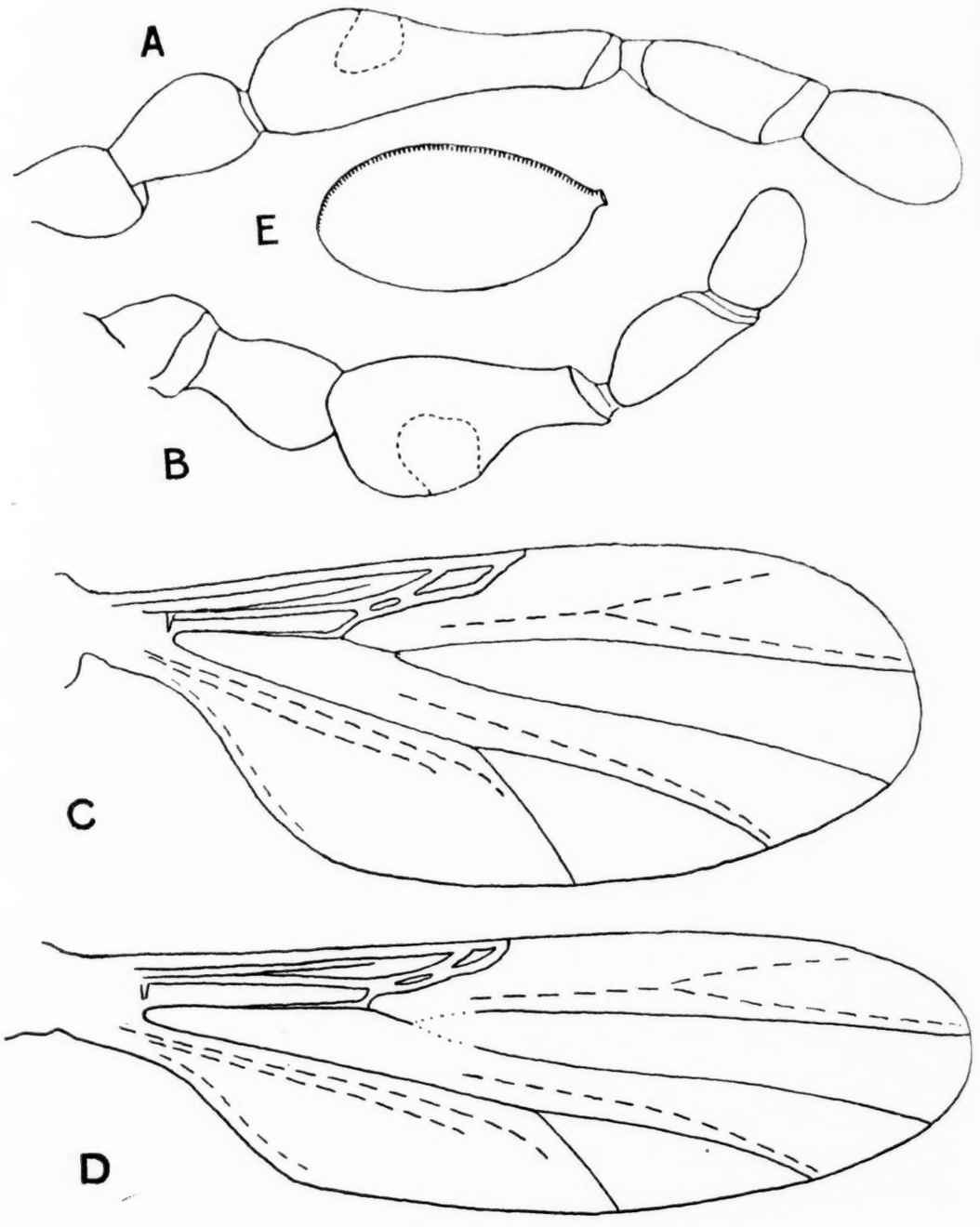

FIG. 1. *Forcipomyia castanea* (Walk.). *A*—Palp of ♂. *B*—Palp of ♀. *C*—Venation of wing, ♀. *D*—Venation of wing, ♂. *E*—Spermatheca. *C* and *D* × *c.* 80; others × *c.* 400.

hairs. Third segment sub-spherical, with a short stalk. Segments 4 to 10 sub-equal, a little longer than broad (11 to 7 units) somewhat flask-shaped. Segments 11 to 15 elongated ; 11 to 14 sub-cylindrical, contracted into a neck just before the apex, sub-equal, about two to three times as long as broad ; the fifteenth stouter, slightly longer, ending in a blunt, club-shaped stylet. The combined length of segments 11 to 15 about equal to that of segments 3 to 10 and a little greater than that of segments 4 to 10, namely, averaging in three specimens approximately 110, 100, and 90 units respectively. In the male segments 3 to 11 bearing spines, those on the basal segments similar to those of the female, but the others smaller ; and large whorls of brown hairs which on the basal segments are almost transverse and situated about the middle of the segments, but become more oblique towards the eleventh segment. The whorls of hairs together form a dense, dark brown plume which reaches nearly to the level of the extremity of the antenna. Torus dark brown, very large, without hairs. Third segment sub-spherical, with a rather long stalk. Segments 4 to 11 sub-spherical to irregularly ovate, becoming narrower and longer towards the eleventh. Segments 12 to 15 elongated, segments 12 to 14 slightly expanded basally and measuring respectively in length and basal breadth about 47 by 8, 28 by 7, and 22 by 5 units ; the fifteenth segment broader, about 32 by 8 units, and ending in a club-shaped stylet. The combined length of segments 12 to 15 slightly greater than that of segments 3 to 10, *e.g.* about 130 to 115 units. *Thorax :* dorsum uniformly dark sepia-brown, but sometimes showing traces of the narrow, pale, admedian lines which in other species are distinct and divide the scutum into three broad longitudinal stripes ; clothed with short, dark brown hairs, intermixed with which are some paler brown hairs. Pleura pale yellowish-brown or cream-buff coloured, sometimes with a dark brown spot beneath the wings. Scutellum dark sepia-brown, bearing in both sexes very numerous long dark brown setae and hairs. Post-scutellum dark sepia-brown, without a central depression. *Wings* (fig. 1, C and D) entirely covered with minute microtrichia and densely clothed with dark brown hairs which are darker and denser on the anterior margin and at the tip of the wing than on the other parts, and some of which are distinctly scale-like ; the extreme base of the wing is pale, buff-coloured, and

there is a small pale, yellow or golden spot about the middle of the anterior border which just covers the junction of the third vein with the costa. Fringe well-developed. In the male the wings are much paler than in the female and the pale spot is indistinct; indeed the area distal to it, which is covered with dark hairs, is often more conspicuous; the wings are also narrower and more translucent. Veins yellowish. Costa reaching to about the middle of the wing. First and third veins forming two cells, the distal one the larger, distinct in both sexes, the proximal one slit-like, barely perceptible in the male. Petiole of the fourth vein longer than the cross-vein. Bifurcation of the fifth vein distal to the bifurcation of the fourth, and in the female at about the level of the end of the costa, but in the male some distance distal to this level. Intercalary veins well-developed, that near the tip of the wing with a long stem. Halteres with creamy-white knobs. *Legs* pale yellowish-brown, clothed with hairs and scales of the same colour which are longer in the male than in the female. Hind femora usually with a narrow sepia-brown band a little distal to the middle; this band varies in size and distinctness to a considerable degree, is sometimes reduced to a mere spot, or may even be entirely absent. *F. incomptifeminibus* (Aust.) must therefore be regarded as only a variety of *F. castanea*. Hind coxae with a dark brown spot on the posterior aspect. Terminal tarsal segments usually slightly infuscated. Femora unarmed. Tibiae with lanceolate scales; fore tibiae with a stout pale yellow apical spur. In both sexes the first tarsal segment slightly shorter than the second on all the legs; the proportions of the hind tarsal segments about 44 : 67 : 41 : 28 : 24. Claws of all the legs equal, small, rather less than half the length of the fifth tarsal segment, curved but not so much as to form a semicircle, and simple in the female, but in the male longer and more slender, more strongly curved than in the female, and with bifid ends. Empodium nearly as long as the claws, with long hairs on both lateral margins. *Abdomen* in the female short and broad, dorsum dark sepia-brown; sides, venter, and cerci paler, yellowish, rather densely clothed with hairs and a few scales which are mostly dark brown; in the male slender, mainly yellowish, but with sepia-brown bands about the middles of the segments which are very variable, but are usually broader on the last three segments, rather sparsely clothed with hairs,

and with a large sepia-brown hypopygium. Spermathecae (fig. 1, E) two, dark brown, highly chitinised, oval or egg-shaped, length about 90μ, greatest breadth about 50μ ; the duct narrow, chitinised for only a short distance (about 7μ) at its commencement.

HYPOPYGIUM (fig. 2). Ninth segment : tergite short and broad, somewhat 'lozenge' shaped, densely clothed with long hairs, posterior margin rounded, with protruding backwards beyond it, a membranous extension bearing on each side a long hair and a well-developed, slightly chitinised process, furnished with two long and two short hairs ; sternite rather broad, not excavated in the middle line posteriorly, covered by long hairs. Forceps : side-pieces large,

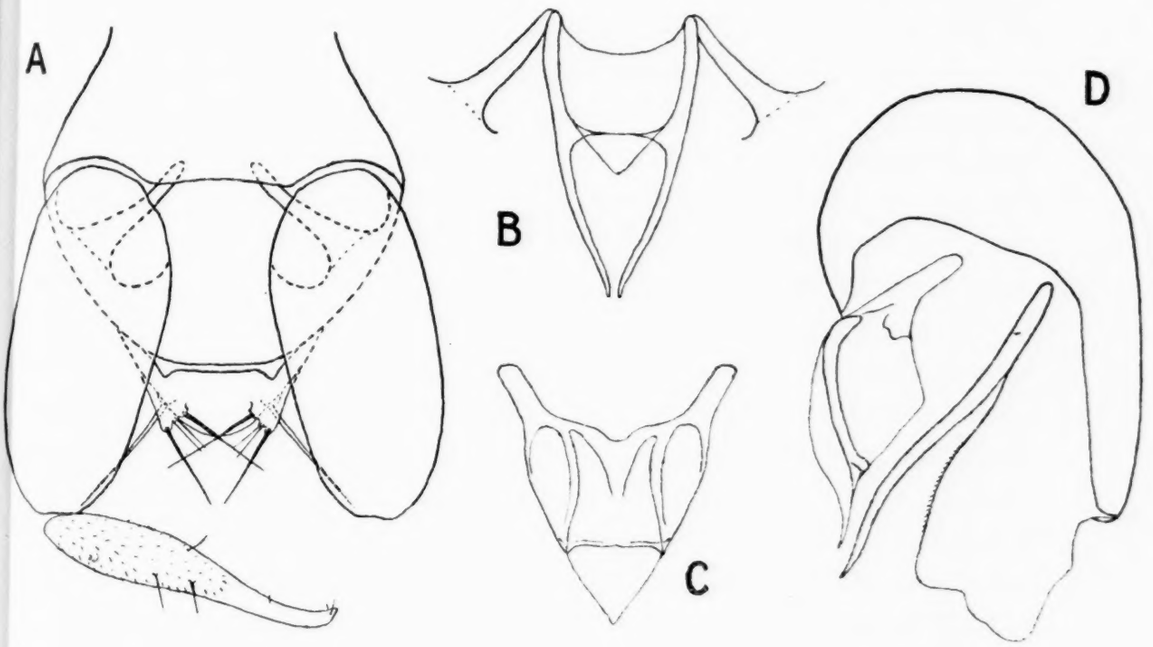

FIG. 2. *Forcipomyia castanea* (Walk.). ♂ : Diagrams of hypopygium. *A*—Ninth segment and forceps, ventral view. *B*—Harpes, ventral view. *C*—Aedoeagus, ventral view. *D*—Lateral view to show the relative positions of harpes and aedoeagus.

twice as long as broad, straight, densely clothed with hairs some of which are very long, prolonged at its proximal end dorsally as a long rod-like process which articulates with the harpe ; claspers similar in form to those of *Culicoides*, about four-fifths the length of the side-pieces, highly chitinised at the distal end, pubescent over the basal half and bearing a few larger hairs. Harpes long, almost straight, rods directed backwards and a little ventrally, moderately well-chitinised, tapering at the apex to sharply pointed tips, their

basal halves united by a thin sheet of chitin which is prolonged posteriorly and medially as a triangular process. Aedoeagus short and very broad, the greater part of it thinly chitinised, terminating posteriorly in a triangular membranous process.

The early stages of *F. castanea* were found by us in 1920 at Accra and Nsawam in rotting fibrous matter at the bases of banana stumps, and were also procured by isolating gravid females taken on the windows of the laboratory in tubes containing rotting vegetable matter. In one such tube in which a single female had been imprisoned on the 15th November, 1920, larvae were first seen on the 23rd (at which time they were already about 2 mm. long), pupae on the 28th, and adults of the new generation on the 30th. From this tube were obtained three adults, fifteen pupae, and thirteen larvae ; the original female had therefore laid thirty-one eggs at least. At this time a full description of the larva and pupa was prepared but was not published, and now, as Saunders (1924) has recently given us a most able and detailed study of the anatomy of the early stages of *Forcipomyia* it is possible to curtail it and in some respects modify it.

PUPA (fig. 3, A to E). The duration of the pupal period is about two days. The larvae pupate on the surface of the medium in which they are living, and the pupae are at first a creamy-white colour but rapidly darken. If left undisturbed, the pupae do not change position appreciably before hatching ; if immersed in water, however, they move their abdomens actively in a vigorous but rather ineffective attempt to rescue themselves. Length about 3·5 mm., greatest breadth about 0·7 mm., well-chitinised, the posterior half of the abdomen loosely enveloped by the larval pelt. The integument is shagreened and bears numerous small tubercles covered with coarse squamose spines, and sometimes armed with short spines or bristles.

Cephalo-thorax relatively large and broad, extended posteriorly over the middle of the first abdominal segment in the form of a relatively long and narrow process. Respiratory trumpets rather short (about 260μ), with a short broad stem and a large expanded distal extremity. The main tracheal trunk is wide, straight in the proximal part of the trumpet, and curved like a hook in the expanded distal part ; at the point where the trumpet begins to expand is

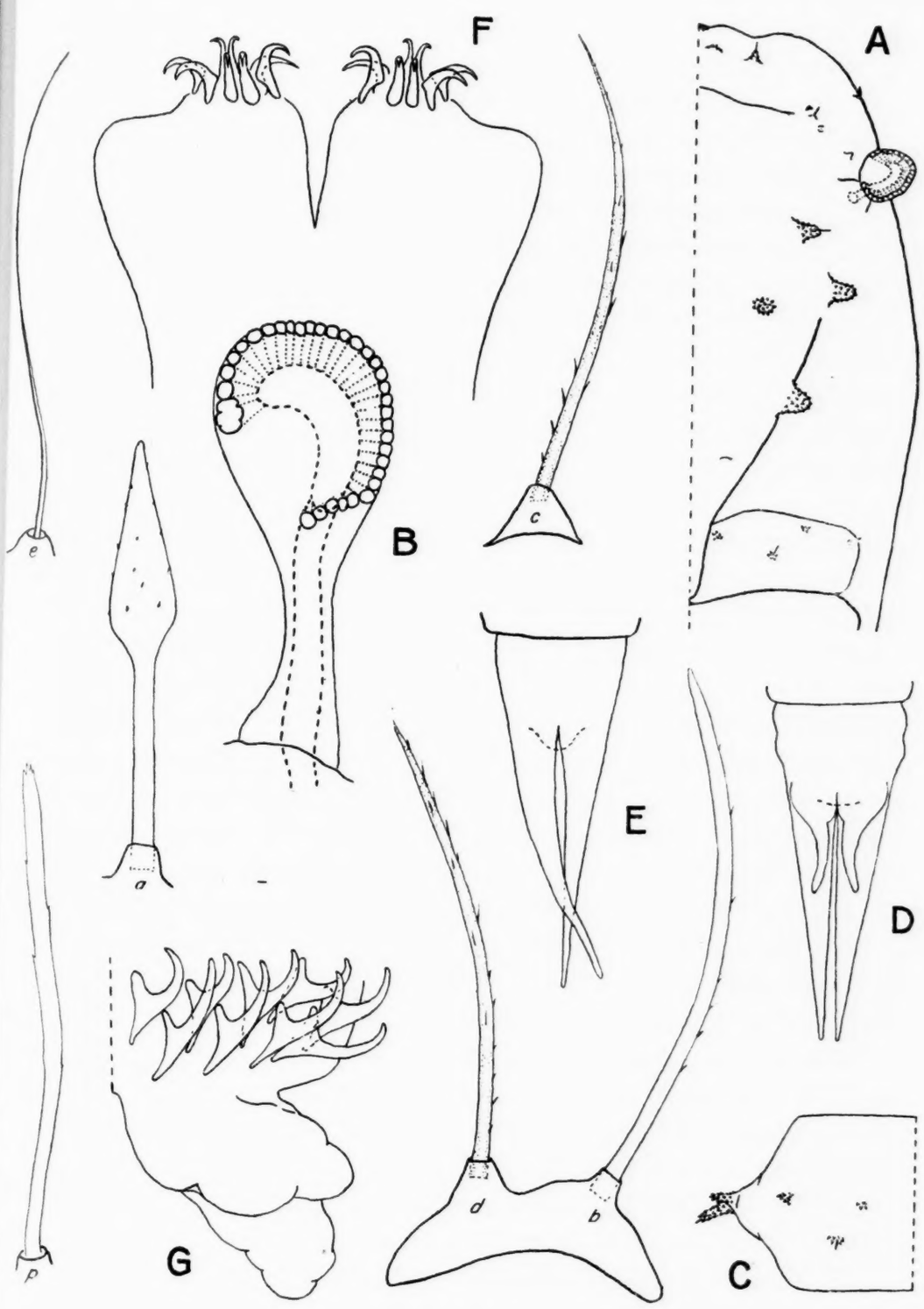

FIG. 3. *Forcipomyia castanea* (Walk.). Pupa: *A*—One half of the dorsal part of the cephalothorax showing the chief tubercles, *B*—Respiratory trumpet, *C*—One half of the fourth abdominal segment, dorsal view, showing the tubercles, *D*—Posterior extremity, ♂, dorsal view, *E*—Posterior extremity, ♀, dorsal view. Larva: *F*—Prothoracic pseudopod, ventral view, *G*—Anal blood-gill and hooks on one half of the posterior extremity of the body, ventral view; *p*, *a*, *b*, *c*, *d*, and *e*—macrochaetae lettered as by Saunders. *A*, *C*, *D*, and *E*, × *c.* 80; others × *c.* 265.

a small posterior diverticulum. From the whole length of the external border of the tracheal trunk in the distal expansion arises a fan-like arrangement of short branches (the number varying greatly, certainly from twenty-three to fifty-four), whose distal extremities form two-thirds of a circle (open only at the proximal side) which is bridged by a delicate granular membrane. The wing and leg cases are attached to the sides and extend backwards on the ventral aspect as far as the anterior border of the third abdominal segment. There are a few tubercles on the cephalo-thorax, all of them rounded or conical prominences, and most of them covered by squamose spines. The following may be distinguished. Situated on the operculum are three low, rounded tubercles which are unarmed; one, the smallest, in the middle line a little behind the anterior margin of the cephalo-thorax, and two, one on each side, at the anterior margin. The latter possibly correspond with the anterior marginal tubercles of *Culicoides*. Anterior dorsal: a small tubercle bearing a short spine, situated a little behind and external to the anterior marginal tubercle. Anterior dorso-lateral: a small tubercle, unarmed, situated just in front of and external to the base of the respiratory trumpet. A little anterior and internal to this tubercle are three other small tubercles arranged in an antero-posterior row, the middle one bearing a short spine and the other two unarmed. Laterally or ventro-laterally, a little posterior to the respiratory trumpet, are two or three small elevations which are unarmed. Dorsal tubercles: situated on each side of the central portion of the thorax are four relatively large, conical, but not finger-like tubercles arranged in the form of a diamond; the anterior one bears a short spine, the other three are unarmed. There are traces of other unarmed tubercles rather more posteriorly, just anterior to the first abdominal segment, but they are very indistinct. No tubercles could be distinguished on the ventral aspect of the cephalo-thorax; the ventro-median is represented by a minute hair.

Abdomen directed straight backwards and partly enclosed in the larval pelt. It consists of nine well-developed segments, and is broad at the base, narrowing gradually but rather rapidly towards the apex. The first segment is short and narrow, the second deep and broad, the ninth elongated, and the intermediate segments rather broad and short, and narrowing successively as the posterior

extremity is approached. The last segment terminates in two long, straight, nearly parallel, lateral processes which taper to pointed extremities; in the male this segment is furnished also with two shorter, finger-like, dorsal processes. The abdominal tubercles are covered with squamose spines and are mostly small; on a typical segment, for example the fourth, they are arranged as follows on each side. On the dorsal surface are three tubercles, two admedian and one dorso-lateral; the admedian tubercles are small, the anterior one unarmed, the posterior one, which is situated rather more externally, bearing a small spine; the dorso-lateral tubercle is small and unarmed, it lies at about the same level as the anterior admedian tubercle. At the side are two relatively large, prominent, finger-like tubercles, the one dorsal to the other, situated about the middle of the segment, and bearing minute spines; the more ventral of the two is the larger. On the ventral surface only a small double tubercle appears to be present, this is situated about the middle of the segment and bears two small spines. The size of the abdominal tubercles diminishes as the posterior end is approached; they may be almost obsolete and represented only by one or two squamose spines on the distal segments. The arrangement of the dorsal tubercles described above applies only to segments 2 to 5, on segments 6 to 8 the tubercles are almost completely absent, being replaced by a few socket-like marks and two or three small spines one of which, situated in a submedian position, is rather strongly developed. The ventral tubercles are recognisable as such on segments 3 to 5 only, on segments 6 to 8 they are represented by two minute spines. On the first segment there are four small tubercles, and two socket-like marks; on the last segment no definite tubercles are discernible, but laterally are three insignificant elevations.

Larva (fig. 3). The body of the young larva has a dull white colour and is semi-transparent; when more mature it is darker, chiefly owing to the dark brown intestinal contents showing through the cuticle. The head is dark brown. The larva is fairly active and browses on the surface of damp vegetable débris. In this situation it is not easily seen, unless the light catches the tips of the dorsal spines and shows them as a long double row of pale, glistening points. In progression the head is used freely. The anal blood-gills are not easily seen in the living larva and are retracted in dead specimens

unless they have been killed and fixed by hot (65° C.) solutions. When about to pupate, the larva ceases to browse, settles in some chosen spot on the surface of the medium, and becomes whiter and more opaque; other changes that may be noted are a thickening of the three anterior body segments due to the development of the imaginal discs, and a retraction of the eyes. The duration of the larval period is about ten days.

Length when mature, 4 mm. to 4·5 mm. *Head* strongly chitinised, almost black anteriorly, showing a colour pattern somewhat similar to that of *F. picea* in Saunders' figure. Antennae of the usual form, short (about 90μ) and straight, and bearing at the end a short, pointed process. The paired sensory hairs *p.* and *q.* of Saunders are long, stout, slightly curved, feebly chitinised, somewhat similar to those of *F. picea* but slightly hairy or serrated. The other hairs of the head apparently similar to those of *F. bipunctata*. Mouth parts similar to those of *F. bipunctata*: mandibles with three large teeth at the distal end.

Body with cuticle covered with spicules. Armature of bristles and spines on the abdominal segments as follows. A pair of long spear-shaped admedian dorsal spines (macrochaeta *a* of Saunders) arising from tubercles which in mature larvae are connected together across the back by a narrow chitinous band; two pairs of long, slightly curved, dorso-lateral setae (*b* and *d*) arising from large double tubercles; a pair of setae (*c*) similar to *d* but longer, arising from conical tubercles; and two pairs of long, stout, simple, ventro-lateral hairs (*e* and *f*) arising from small tubercles. The spear-shaped dorsal spines are about 200μ long, the blade-like distal portion measures about 25μ at its broadest part, has a long point, and is sparsely covered with spicules; the length of the shaft is only slightly greater than that of the blade-like portion. The description of these spines as spear-like is scarcely appropriate, as the distal portion is not flat but is cone-shaped; in lateral view, however, they appear spear-like, and as this descriptive term has been used before, it is again used here. There are twelve pairs of these spines, the first pair being rather small, and the last modified, the tubercles from which they arise being broadly fused and the distal ends of the spines being only slightly broader than the shafts. The two dorso-lateral setae (*b* and *d*) arise the one slightly more dorsally than the

other; the former (*b*) is not highly chitinised, is very sparsely clothed with barbs, is rather blunt-ended, and is similar to the head hairs *p* and *q*; the latter (*d*) is shorter, highly chitinised, dark coloured, well-barbed, and more pointed.

The prothoracic pseudopod is cleft distally but not very deeply, the two parts not widely separated, broad at their extremities, with the crowns of hooks (six strong, and two more delicate) situated on their inner sides. Anal blood-gills bilobed. Posterior pseudopod armed with, usually, nine large hooks in two rows on each side of the middle line. Malpighian tubules apparently three.

Egg. The eggs are a dull white colour, smooth, ellipsoidal or whetstone-shaped; length about 205μ, middle breadth about 78μ. When laid they apparently adhere by their sides; they may sometimes be seen thus, forming a chain, whilst still attached to the body of the female.

Gold Coast: Accra, numerous specimens, both males and females, taken on the windows of the laboratory in the evening at all times of the year. Numerous specimens also reared from eggs laid by females in captivity, and hatched from rotting banana fibre. Nsawam, reared from vegetable matter from a rot-hole in a tree.

FORCIPOMYIA BIANNULATA, sp.n.

Length of body (average), 2 mm.; length of wing, 1·3 mm.; greatest breadth of wing, 0·3 to 0·5 mm. In the male the wings are relatively longer and narrower than in the female. With narrow scales.

Head dark brown. Eyes bare, broadly contiguous above in both sexes, the facets separated by a narrow line. Clypeus, proboscis, and palpi dark brown. Palpi similar to those of *Forcipomyia castanea*. *Antennae* brown. In the female segments 3 to 10 bearing stout, curved spines, about as long as the segments, colourless, with rather blunt ends; and whorls of about seventeen hairs. The flagellum segments sub-equal, forming an almost continuous series, the fifteenth, however, slightly longer than the rest; segments 3 to 10 somewhat flask-shaped, segments 11 to 14 with a distinct 'collar,' and the last segment ending in a small blunt stylet. The combined length of segments 11 to 15 less than that of segments 3 to 10,

or 4 to 10, *e.g.* in one specimen measuring 77 units as compared with 118, or 101 units. In the male similar to those of *F. castanea*, but the last segment perhaps a little shorter, the lengths of the five terminal segments in one male being 12, 47, 26, 20, and 25 units respectively. The combined length of segments 12 to 15 equal to that of segments 3 to 11. *Thorax* uniformly dark brown dorsally, densely clothed with yellowish hairs. Pleura paler, golden-brown, paler in the male than in the female, with a darker brown spot immediately under the base of the wing. Scutellum and post-scutellum dark brown, the former bearing numerous long, yellowish bristles and shorter hairs which are more abundant in the female than in the male. *Wings* similar to those of *F. castanea*, densely clothed with dark brown hairs and narrow scales, and with a small pale, yellowish spot at the distal end of the costa enveloping about two-thirds of the distal radial cell. The wings in the male are narrower than in the female, less hairy, but with a distinct pale spot, and some infuscation in the neighbourhood of the intercalary vein and of the rami of the fourth and fifth veins. Venation similar to that in *F. castanea ;* but the first and third veins are fused basally so that the first radial cell is obsolete ; the second radial cell, however, is well-formed. Halteres with cream-coloured knobs and almost colourless stems. *Legs* yellowish-brown, terminal tarsal segments somewhat infuscated. Hind femora with a broad dark brown apical band which completely encircles the limb, hind tibiae with a similar band covering the basal third, knees not infuscated. The development of the bands on the hind legs is subject to some degree of variation. Legs moderately hairy and bearing also narrow scales, a few of the hairs, such as two on the ventral aspect of the first tarsal segment, being exceptionally long. The middle and hind tibiae of the female bear also one or two peculiar short, hastate hairs or spines (fig. 4, D) : these spines are not present in the male. In the male the first tarsal segment about equal to the second on the fore legs, but shorter (about three-quarters the length) on the middle and hind legs ; in the female the first tarsal segment about equal to the second on the middle and hind legs, longer (about one-third longer) on the fore legs. Claws and empodium similar to those of *F. castanea*. *Abdomen* in the female dark brown dorsally, paler brown ventrally and at the sides, clothed with dark and light brown

hairs and narrow scales ; in the male paler, light brown, with broad dark brown bands on the segments which are broadest ventrally, and larger and more complete towards the posterior extremity ; clothed with rather sparse and long hairs. Hypopygium dark brown. Spermathecae two, dark brown, highly chitinised, more or less oval, often unequal (in one extreme example measuring 68μ by 65μ and 133μ by 87μ respectively) ; the commencement of the duct chitinised for only a short distance (about 4μ).

HYPOPYGIUM (fig. 4, A to C) similar to that of *F. castanea* but more highly chitinised and more deeply infuscated. Ninth segment similar to that of *F. castanea*, but sternite less hairy. Forceps similar to those of *F. castanea*, but darker, the tips of the claspers being dark brown ; side-pieces clothed with strong hairs which, however, are not very long. Harpes long, slender rods tapering to

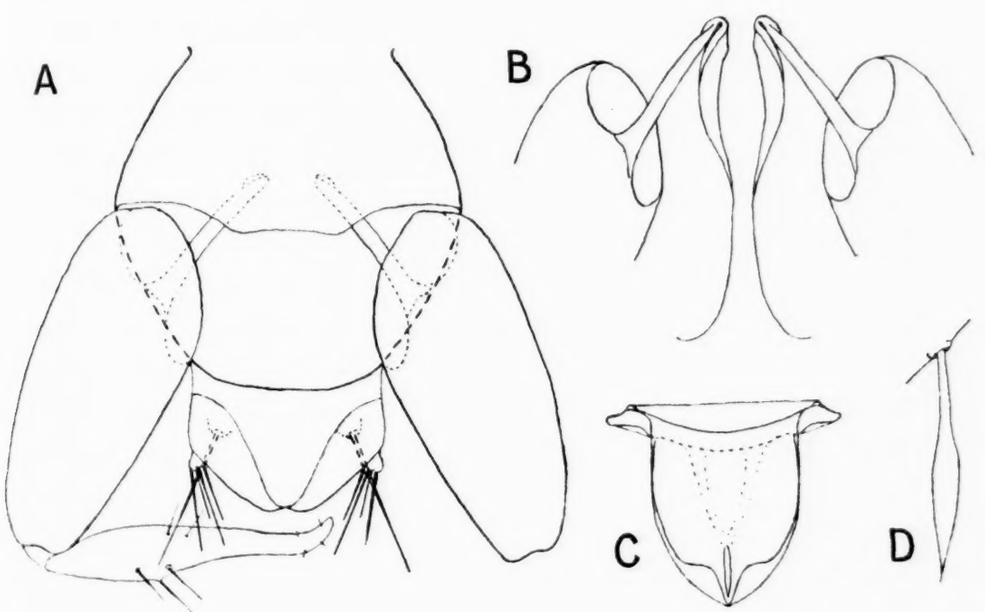

FIG. 4. *Forcipomyia biannulata*, sp.n. ♂ : *A*—Ninth segment and forceps, *B*—Harpes, *C*—Aedoeagus, all ventral views. ♀ : *D*—Hastate tibial hair.

filiform extremities, similar to those of *F. ashantii*. Aedoeagus broad and shield-like in ventral view, somewhat resembling that of *F. castanea* but differing in detail and more highly chitinised.

GOLD COAST : Accra, numerous specimens, both males and females, taken in the evenings on the windows of the laboratory at all times of the year, but more abundantly during the second half.

NIGERIA: Afikpo, 2.6.1910, 1 ♀ (Dr. J. J. Simpson); Lagos, June, 1921, 1 ♀ (Dr. H. A. Foy).

NYASALAND: South-east shore of Lake Nyasa, March, 1910, 1 ♂ (Dr. S. A. Neave).

This species resembles *F. castanea* but may be readily distinguished from it by the presence of a dark brown basal band on the tibiae of the hind legs.

FORCIPOMYIA AURIPES, sp.n.

Length of body (one male), 2·9 mm.; length of wing, 1·8 mm.; greatest breadth of wing, 0·4 mm. A large, dark brown species with dark brown femora and tibiae and yellowish tarsi.

Head dark brown. Eyes bare; broadly contiguous above, but actually very narrowly separated. Clypeus and proboscis brown. First and second segments of the palp brown, other segments missing. *Antennae* incomplete in the single specimen examined. Basal segments of the flagellum brown apically, yellowish basally, the line of insertion of the whorl hairs separating the two colour zones; plume dark brown with a paler brown tip. The length and greatest breadth of the eleventh and twelfth segments about 20 by 9, and 78 by 8 units respectively. Segments 13 to 15 missing. *Thorax* almost uniformly dark brown, but showing traces of the three longitudinal stripes on the dorsum so common in the genus; pleura slightly paler brown than the dorsum. Hairs rather sparse, no scales. Scutellum and post-scutellum dark brown; the former bearing about twenty bristles and hairs. *Wings* long and narrow, pale brown, closely covered with minute microtrichia of the usual form and densely clothed with decumbent hairs; bearing also on the costa and first and third veins long lanceolate scales. No bare areas along the veins. Costa reaching beyond the middle of the wing (65 : 101 units), to the distal third. First and third veins contiguous basally, the proximal cell obsolete or a mere slit, the distal one long and narrow. Bifurcation of the fifth vein proximal to the end of the costa, at about the level of the base of the distal radial cell; anterior ramus reaching the margin of the wing a considerable distance beyond the level of the end of the costa. Hairs of fringe with rather long fimbriae, sub-plumose. Halteres darkish

brown, knobs containing a white pigment. *Legs* with dark brown femora and tibiae, yellowish tarsi. Scales present on the femora and distal segments. First tarsal segments two-and-a-half, two, and one-and-a-half times as long as the second on the fore, middle, and hind legs respectively. Claws about half the length of the fifth tarsal segment ; empodium as long as the claws, hairy. *Abdomen* dark brown, especially posteriorly, and in the single specimen examined containing much actually black pigment. Hairs on the posterior part of the abdomen long, especially those on the ventral surface. Scales present on the dorsal surface.

HYPOPYGIUM (fig. 5). Ninth segment very dark brown, both tergite and sternite well-clothed with dark brown hairs of moderate

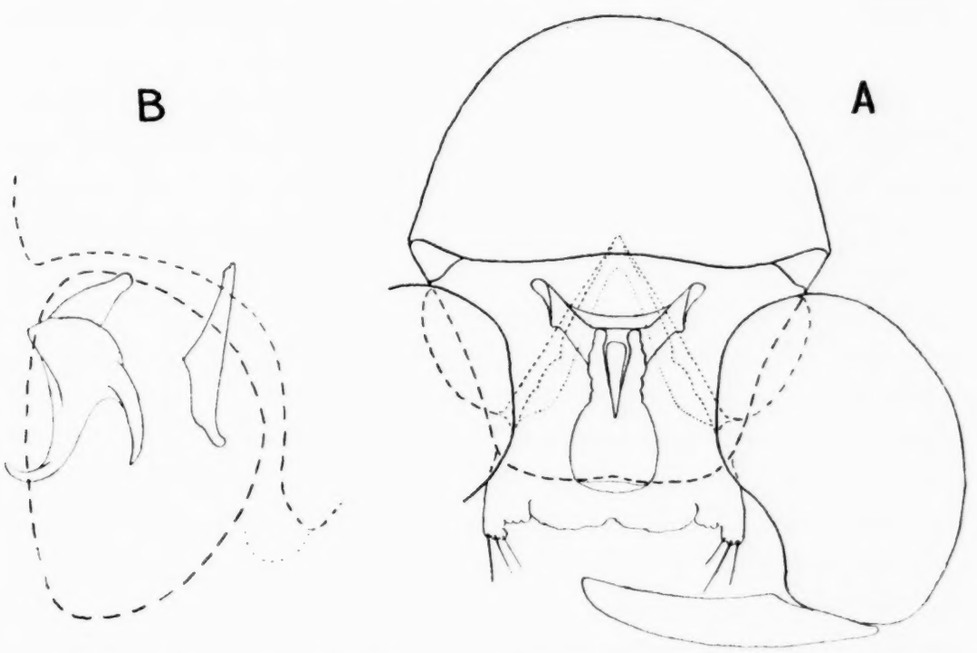

FIG. 5. *Forcipomyia auripes*, sp.n. ♂ : Diagrams of hypopygium. *A*—Ventral view, *B*—Lateral view to show the relative positions of the aedoeagus and the dorsal process arising at the base of the side-piece. × *c.* 265.

length ; sternite not notched in the middle line posteriorly. Forceps : side-pieces broad, uniformly very dark brown, clothed with numerous dark hairs some of which are of great length and others very stout and strong ; claspers dark brown, shaped like the blade of a scythe, pubescent to the tips. Harpes : the rod-like structures which arise dorsally at the bases of the side-pieces converge anteriorly and meet in a sharp point ; these rods are of dense chitin, but are extended internally into a narrow strip of delicate chitin. Aedoeagus

in ventral view showing a broad basal arch which is well-chitinised, and a posterior extension of delicate chitin with a rounded, slightly everted end.

GOLD COAST: Accra, 1920, 1 ♂, taken in the laboratory.

The position of this species is somewhat obscure. We have placed it in the Genus *Forcipomyia* because many of its characters are those of that genus, but it should be noted that the genitalia are not of the common type, and that the length of the costa and the form of the radial cells are unusual and more closely resemble those of the Genus *Lasiohelea*.

FORCIPOMYIA SQUAMIPENNIS, sp.n.

Length of body (both sexes), about 2·0 mm.; length of wing in the female, 1·4 mm., in the male 1·3 mm.; greatest breadth of wing in the female 0·4 mm., in the male 0·3 mm.

Head dark brown; occiput clothed with long, dark brown hairs intermixed with which are numerous rather short, striated scales. Eyes bare, broadly contiguous above in both sexes, the facets separated by a narrow line. Clypeus, proboscis, and palpi dark brown. Clypeus bearing about sixteen hairs. Palpi similar in both sexes; second, fourth, and fifth segments sub-equal, the last two rather broadly united, third segment longer than the fourth and fifth together, inflated in its middle third, and furnished with a shallow and not unusually large sensory pit. *Antennae* (fig. 6, c) brown. In the female segments 3 to 10 bearing long, slender spines, not much thicker than the hairs, as long as the segments, and tapering to rather sharp ends; and whorls of about twelve hairs. All the flagellum segments bear also short, and moderately short, spines. First segment rather large, petiolate, bearing numerous stout, dark brown, pubescent hairs, the longest of which project beyond the distal border of the torus. Torus sub-spherical, darker brown than the flagellum, bearing about a dozen hairs. Third segment with a quite short stalk. Segments 4 to 10 flask-shaped, almost sub-equal, length from about twice to two-and-a-half times the greatest breadth. Segments 11 to 15 sub-cylindrical, 11 to 14 sub-equal, about three times as long as broad, with a distinct 'collar'; the last segment

a little longer, about four times as long as its basal breadth, ending in a blunt stylet. The combined length of segments 11 to 15 (128 units) rather less than that of segments 4 to 10 (145 units). In the male segments 3 to 11, generally, similar to those of *F. castanea*, the eleventh measuring about 16 units by 8; plume large, with its distal half tawny brown. Segments 12 to 15 elongated, their lengths measuring in one specimen 57, 34, 28 and 28 units respectively, the last segment ending in a blunt stylet. The combined length of segments 12 to 15 (147 units) almost equal to that of segments 3 to 11 (142 units), and greater than that of segments 4 to 11 (119 units). *Thorax* uniformly darkish brown; well-clothed dorsally with light brown hairs and narrow scales, and with longer and darker hairs marginally and posteriorly. Pleura brown, paler than the dorsum. Scutellum dark brown, bearing an irregular submarginal row of about twelve to fifteen large, pale brown bristles, and numerous (twenty-five to fifty) small hairs and scales; the number of bristles and hairs in the male is smaller than in the female. Post-scutellum dark brown, bare. *Wings* in both sexes covered with the usual minute microtrichia and densely clothed all over with small, rather broad scales. In the female dark grey, with a number of rather ill-defined, cream-coloured spots arranged in much the same manner as those on the wings of *Culicoides*. Two pale spots are situated on the anterior half of the wing, the one just beyond the junction of the third vein with the costa, and the other, a large, diffuse spot, upon the apical fourth; there is also some indication of a pale spot covering the cross-vein, and the base of the wing is somewhat pale. Along the margin on the posterior half of the wing are four ill-defined pale spots, one each, between the rami of the fourth vein, between the fourth and fifth veins, between the rami of the fifth vein, and in the anal angle. The wing is darkest anteriorly, especially at the junctions of the first and third veins with the costa, and is clothed with scales, those upon the cream-coloured spots being of a yellow colour, those upon the darker portions of the wing being dark grey or dark brown. The scales are especially dense, broad, and dark upon the costa and the first and third veins. In the male the wings are narrower, less densely covered with scales, and a lighter colour, so that they appear to be pale, with darker markings, and not the reverse as in the female. Fringe long, composed of lanceolate

hairs which are delicately fringed. Costa reaching to about the middle of the wing. First and third veins fused basally, the proximal cell obsolete ; second radial cell (fig. 6, A) large, about three times as long as broad (external dimensions). Petiole of fourth vein longer than the cross-vein ; proximal ends of the rami almost obsolete. Bifurcation of the fifth vein in both sexes a little proximal to the level of the end of the costa. Halteres with dull white knobs. *Legs* in both sexes conspicuously banded, yellow and dark brown ; femora, tibiae, and tarsi densely clothed with scales in addition to hairs. Coxae of all the legs dark brown. Femora with a broad dark brown band at base and apex, the bands being larger in the female than in the male, and the apical band in the male sometimes divided into two parts. Fore and middle tibiae with broad dark brown basal and apical bands each covering about one-third of the segment ; hind tibiae with the basal third dark brown and the apical two-thirds yellow with a narrow dark brown band in its middle. The knees, however, are usually yellow. First tarsal segment with the basal two-thirds dark brown and the apical third yellow ; second, third, and fourth segments more or less infuscated dark brown, in the middle ; fifth entirely yellow. Middle tibiae with a transverse row of apical spine-like hairs, some of which may be nearly as long as the first tarsal segment. In both sexes the first tarsal segment on the fore legs is longer than the second (about 4 : 3), on the middle legs slightly shorter (about 11 : 12), and on the hind legs distinctly shorter (about 5 : 7). Claws small, less than half the length of the fifth tarsal segment ; apparently not bifid in the male. Empodium about as long as the claws, hairy. *Abdomen* in the female uniformly dark brown, densely clothed with broad scales and with dark and light brown hairs, a tuft of hairs on the posterior extremity being especially long. Cerci pale brown. In the male pale, yellowish, longer and narrower than in the female, with dark brown bands near the posterior margins of the segments, which are broadest ventrally and towards the posterior end of the body ; the dorsal aspect of the proximal half of the abdomen almost entirely yellow, and the ninth segment entirely dark brown. Abdomen well-clothed with scales and hairs, the latter particularly long and numerous on the ventral surface. Spermathecae (fig. 6, D) two, very highly chitinised, sub-equal, pyriform, with a rather long conical portion

leading to the duct; greatest breadth about 45μ to 50μ, and total length about 80μ to 85μ.

HYPOPYGIUM (fig. 6, E and F). Ninth segment dark brown; tergite very short, sternite with numerous hairs and scales. Forceps: side-pieces with dark brown apices and paler, yellowish bases; claspers rather densely chitinised basally, their ends somewhat expanded, spatulate, but not hatchet-shaped. Harpes very highly chitinised, shaped like strong hooks, crossing in the middle line, with their extremities bent ventrally and tapering to a point.

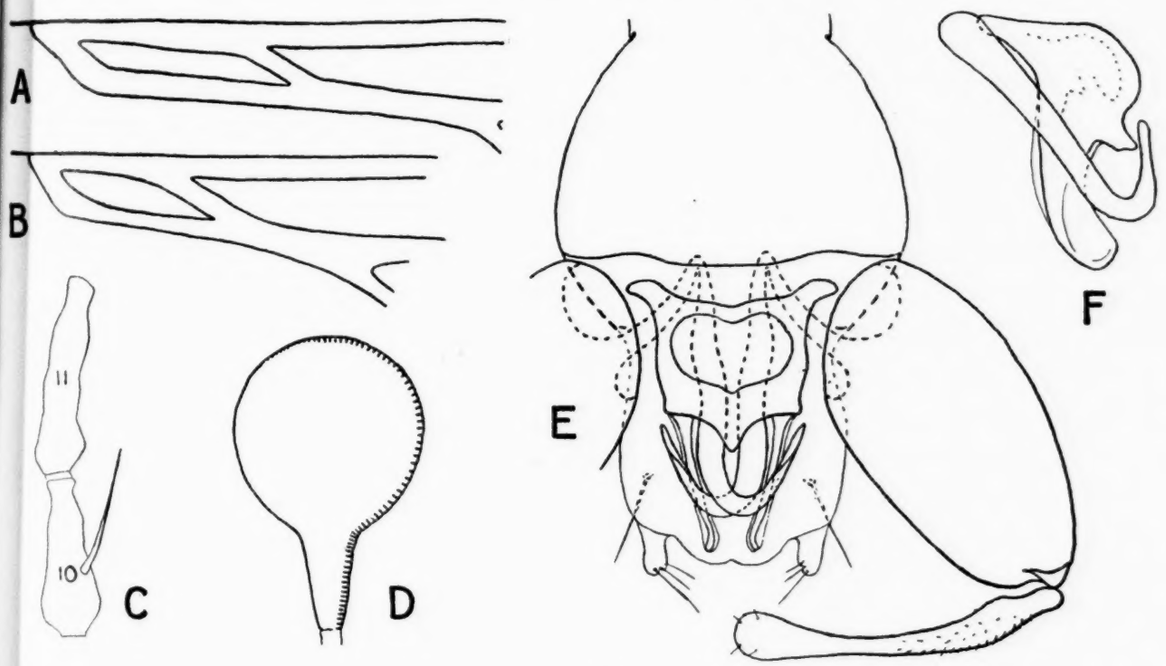

FIG. 6. *Forcipomyia squamipennis*, sp.n. *A*—Distal radial cell of wing of ♂, *C*—Tenth and eleventh segments of antenna of ♀, *D*—Spermatheca of female, *E*—Hypopygium of ♂, ventral view, *F*—Harpes and aedoeagus, lateral view. *Forcipomyia lepidota*, sp.n. *B*—Distal radial cell of wing of ♂. *D* × *c.* 400; others × *c.* 265.

Aedoeagus very highly chitinised in parts, having a ventral shield-like portion, the posterior edge of which is almost black and is produced into a median and two lateral projections, and on each side a little more dorsally, a strong, ventrally curved, rod-shaped structure.

GOLD COAST: Accra, 1920, ♂♂ and ♀♀; Aburi, 1912-13, 2 ♀♀ (W. H. Patterson).

It is not improbable that *F. chrysolopha* (K.) is identical with this or one of the three following species, but this fact can only be determined by a re-examination of the type materials, particularly the genitalia of the males.

FORCIPOMYIA LEPIDOTA, sp.n.

This species resembles the preceding one, *F. squamipennis*, in almost every respect excepting in the structure of the hypopygium of the male. The only points of distinction, and some of these may not be of any importance, since they are in characters which in other species are liable to variation, that we are able to detect after a careful comparison are as follows : The distal end of the antennal plume is pale, but not tawny ; the terminal segments (11 to 15) of the antenna of the male measure in length (average of four specimens) about 14, 37, 22, 16, and 22 units respectively, the fourteenth segment, therefore, about one-fourth shorter than the fifteenth, and not about equal to it as in *F. squamipennis*. Scales on the wings smaller than in *F. squamipennis*, those on the surface of the wing narrower, more like lanceolate hairs. Fringe hairs lanceolate, but apparently simple. Distal radial cell (fig. 6, B) shorter. Bifurcation of the fifth vein slightly distal to the level of the end of the costa. Femora with dark bands broader than in *F. squamipennis*, occupying the greater part of the segment. Hind tibiae with basal dark-brown band somewhat narrower, and the second dark brown band somewhat broader than in *F. squamipennis*, so that the segment is divided into four areas of about equal length, alternately dark brown and yellow. The structure of the hypopygium is, however, quite unlike that of *F. squamipennis*.

HYPOPYGIUM (fig. 7, A and B). Ninth segment dark brown, not so densely covered with hairs as in *F. squamipennis*. Forceps : side-pieces dark brown, slightly paler brown or yellowish at the apex ; claspers pale brown, rather delicately chitinised, with the distal extremity greatly expanded. Harpes apparently partly enfolded in the membranous portion of the aedoeagus, and difficult to see clearly in a fresh specimen. After treatment with caustic potash appearing as long, stout, strongly chitinised rods, tapering distally, and ending rather bluntly. Aedoeagus a complicated structure only partly, and not very highly chitinised ; in ventral view the most characteristic features are a basal, stirrup-like portion with a median ventral process, and a distal, shuttle-like structure.

GOLD COAST : Accra, 1920, numerous specimens ; Sekondi, July 1922, 3 ♂♂, 1 ♀, in a bungalow (Dr. J. F. Corson).

Owing to the similarity of this species to several others obtained at Accra which, apart from the structure of the hypopygium of the male, are almost, if not quite, indistinguishable, it has not been possible to decide with certainty which, if any, of the females of this type collected by us should be associated with it. A large number of females were collected with the males, all of which are practically identical, apart from variations in size which do not appear to be of importance. We are unable to separate them into different

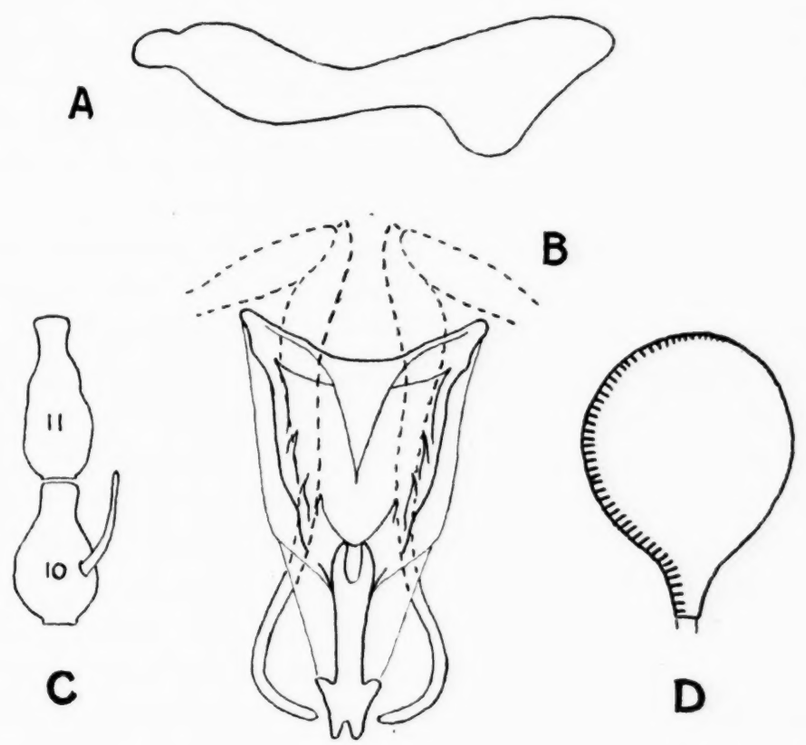

FIG. 7. *Forcipomyia lepidota*, sp.n. *A*—Outline of clasper of ♂, *B*—Diagram of harpes and aedoeagus of ♂, ventral view, *C*—Tenth and eleventh segments of antenna of ♀, *D*—Spermatheca of ♀. *D.* × *c.* 600; others × *c.* 400.

species as has been done in the case of the males, and it must therefore be left for future investigation to decide whether they represent one or several species. In the meantime a description of the female should be given, and we propose to give it here, because the male *F. lepidota* was abundant in our material and it therefore appears to us probable that the female also was abundant, and because one such female was taken in a bungalow at Sekondi together with three males

of *F. lepidota*, the only species of this group hitherto collected in that locality. It must be clearly stated, however, that with equal or almost equal justice it might be claimed that the description is also that of the females of the two following species (*F. venusta* and *F. pampoikila*), and that we are unable to detect any differential characters of specific importance between the female reared from banana fibre together with a male of *F. venusta*, and therefore presumably the female *F. venusta*, and the female taken at Sekondi, together with three males of *F. lepidota*, and therefore presumably the female *F. lepidota*.

FEMALE. Length of body, 1·3 mm. to 1·8 mm.; length of wing, 0·8 mm. to 1·0 mm.; greatest breadth of wing, 0·3 mm. to 0·4 mm.

Head similar to that of *F. squamipennis*, but hairs on clypeus apparently fewer, five to seven, and third segment of the palp furnished with a deeper sensory pit. *Antennae* (fig. 7, C) brown. Large spines on segments 3 to 10 more or less geniculate, twice as stout as the hairs, shorter than the segments, with blunt ends. Flagellum segments forming an almost continuous series, as in *F. squamipennis*, but more distinctly. Segments 4 to 10 slightly constricted apically, but not so clearly flask-shaped as in *F. squamipennis*, sub-equal, less than twice as long as broad, the length and greatest breadth of segments 4 and 10 being in one example 9 by 7, and 10 by 6 units, respectively. Segments 11 to 15 elongated, sub-cylindrical, 11 to 14 sub-equal, about twice as long as broad (13 by 5 units), the last segment a little longer (17 units), about three times as long as broad. The combined length of segments 11 to 15 slightly less than that of segments 4 to 10, in one specimen 74 to 77 units. *Thorax* similar to that of *F. squamipennis*. *Wings* similar to those of *F. squamipennis*, but scales on the general surface narrower, as in the male. Distal radial cell as in the male *F. lepidota*. Bifurcation of the fifth vein slightly proximal to the level of the end of the costa. *Legs* similar to those of *F. squamipennis*; the hind tibiae adorned as in the male *F. lepidota*. *Abdomen* similar to that of *F. squamipennis*, but the scales rather narrower. Spermathecae (fig. 7, D) two, similar to those of *F. squamipennis* but smaller (average length about 60μ, and greatest breadth about 40μ), and with the proximal conical portion less largely developed.

FORCIPOMYIA VENUSTA, sp.n.

This species resembles *F. lepidota* so closely, that apart from the characters of the hypopygium of the male we are unable to separate the two with certainty. The measurements of the terminal segments (11 to 15) of the antenna of the male averaged in four specimens 15, 44, 26, 20, and 22 units in length respectively. The banding of the hind tibiae is as in the preceding species, *F. lepidota*. The structure of the hypopygium is, however, quite distinctive.

HYPOPYGIUM (fig. 8). Ninth segment dark brown; sternite rather more hairy than in *F. lepidota*. Forceps: side-pieces and claspers similar to those of *F. lepidota*. Harpes long, slender plates of chitin, not very highly chitinised, almost straight, tapering

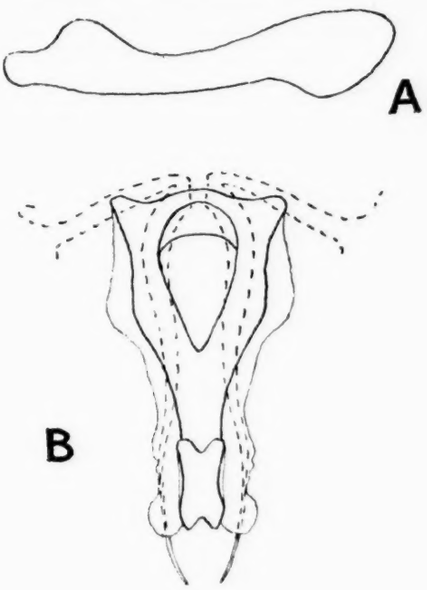

FIG. 8. *Forcipomyia venusta*, sp.n. ♂: *A*—Outline of clasper, *B*—Diagram of harpes and aedoeagus, ventral view.

distally and ending in sharp points. Aedoeagus similar to that of *F. lepidota*, but stirrup-shaped portion more uniformly chitinised, as shown in the figure.

GOLD COAST: Accra, 1920, numerous specimens taken on the laboratory windows; Nsawam, near Accra, 26.5.1920, 1 ♂, 1 ♀, reared from rotting banana fibre.

As in the preceding species, we have not been able to separate with certainty the female of this insect from those of allied species (see *F. lepidota*).

FORCIPOMYIA PAMPOIKILA, sp.n.

This species, like the preceding one, resembles *F. lepidota* so closely that apart from the characters of the hypopygium of the male we are unable to distinguish them with certainty. The measurements of the last five segments (11 to 15) of the antenna of the single male in our possession are approximately 14, 38, 23, 21, and 24 units respectively. The banding of the hind tibiae is similar to that in *F. lepidota*. The hypopygium is characteristic.

HYPOPYGIUM (fig. 9). Ninth segment dark brown, closely covered with hairs both dorsally and ventrally. Forceps: side-pieces dark brown at base and apex, with a paler, yellowish band

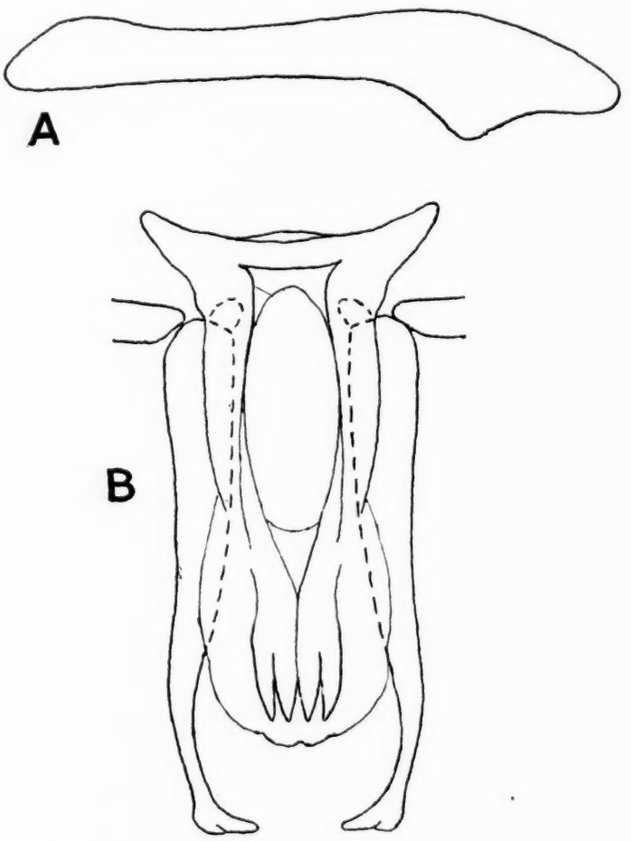

FIG. 9. *Forcipomyia pampoikila*, sp.n. ♂: *A*—Outline of clasper, *B*—Diagram of harpes and aedoeagus, ventral view. × *c.* 400.

about the middle; claspers pale brown, not very highly chitinised, only slightly expanded at the end. Harpes long, slender plates of chitin, almost straight, tapering distally and with ends slightly

expanded and shaped like the head of a duck. Aedoeagus of the usual form but mainly membranous or but feebly chitinised, distal end showing in ventral view four short chitinised processes.

GOLD COAST: Accra, 1920, 1 ♂, taken in the evening upon a window of the laboratory.

FORCIPOMYIA NIGROTIBIALIS, sp.n.

Length of body (one female), 1·6 mm.; length of wing, 1·2 mm.; greatest breadth of wing, 0·4 mm. A very dark brown, almost black, species, with dark brown tibiae on the middle and hind legs; with scales. We have in our possession only a single specimen of this insect, and this unfortunately was partially destroyed by Psocids before our examination was completed, and in consequence our description is in some particulars deficient.

Head very dark brown. Proboscis and palpi dark brown. *Antennae* dark brown, with short dark brown hairs. Basal segments missing. Segments 7 to 10 oval, slightly narrowed anteriorly, sub-equal, about 12 by 7 units; large spines pale brown, stouter than the hairs, a little longer than the segments, almost straight, tapering to pointed extremities; the whorls of about twelve hairs. Segments 11 to 15 more elongated, sub-cylindrical, 11 to 14 sub-equal, about 15 by 6 or 7 units, the last segment a little longer, about 21 units, ending in a short, blunt stylet. *Thorax:* Dorsum very dark brown. Pleura very dark brown above, paler brown below. Scutellum very dark brown; bearing a transverse row of seven large bristles, and about ten smaller bristles and hairs. Post-scutellum very dark brown. *Wings* unadorned, but the anterior margin somewhat darker than the rest of the wing, and the extreme base yellowish; well-clothed with dark hairs and narrow scales. Microtrichia minute. Fringe as usual; long hairs slightly curved, slender, apparently simple. Costa reaching to, or very slightly beyond, the middle ot the wing. First radial cell obsolete or nearly, second well-formed. Bifurcation of the fifth vein at about the same level as the end of the costa. Halteres with yellowish knobs. *Legs* conspicuously marked, well-clothed with hairs and narrow scales, and bearing (especially on the femora and tibiae) large hastate spines with long tapering ends. Fore legs nearly

uniformly light brown. Middle legs with femora and tibiae very dark brown, excepting at the base of the femora and the apex of the tibiae, where they are light brown ; tarsal segments light brown. Hind legs with apical half of femora light brown, basal half very dark brown ; tibiae very dark brown, excepting at the extreme apex, which is slightly tawny ; tarsal segments light brown. First tarsal segment of the middle and hind legs shorter than the second (about 35 : 41 and 40 : 49 units), that of the fore legs about equal to the second (about 36 : 33 units). Claws simple, equal, less than half the length of the fifth tarsal segment. Empodium well-developed, hairy. *Abdomen* very dark brown ; densely clothed with dark brown scales. Spermathecae two, highly chitinised, sub-spherical, sub-equal, diameter about 50μ to 60μ ; the commencement of the duct chitinised for a very short distance, about 4μ.

GOLD COAST : Accra, 1 ♀, taken in the evening upon a window in the laboratory.

FORCIPOMYIA INORNATIPENNIS (Austen).

Length of body, 2·1 mm. to 3·1 mm. ; length of wing, 1·4 mm. to 1·9 mm. ; greatest breadth of wing, 0·4 mm. to 0·7 mm. The size varies greatly in both sexes, and the males are rather longer than the females on account of the size of the hypopygium, and have longer and narrower wings. In different individuals the colour also varies considerably, from brown to very dark brown, and females are usually darker than males. Long, slender, striated scales present.

Head brown to very dark brown ; occiput clothed with yellowish or dark brown hairs. Eyes bare ; broadly contiguous above in both sexes. Clypeus, proboscis, and palpi dark to very dark brown. Clypeus bearing about six to eight dark brown hairs. Mouth parts similar to those of *F. castanea*, but the mandibles of the female more slender and pointed at the ends, and armed with more numerous teeth (about fifty to sixty), those in the middle of the series being rather large ; teeth on the maxillae also more numerous, about twenty-five. Palpi (fig. 10, A and B) in both sexes with second and fourth segments sub-equal, fifth small, not much more than half the length of the fourth, and third much the longest, twice as long as the fourth. In the female the third segment in its middle three-fourths is greatly inflated on its inner aspect, and is furnished with a very

large, oval, sensory pit with a relatively small anterior opening near which, on the surface of the segment, are a number of sensory hairs; in the male the third segment is slender, inflated to a lesser extent in its middle third, and furnished with an oval sensory pit of moderate size. *Antennae* brown to darkish brown. In the female segments 3 to 10 bearing moderately stout, slightly curved spines, as long as the segments, colourless, with pointed ends; and whorls

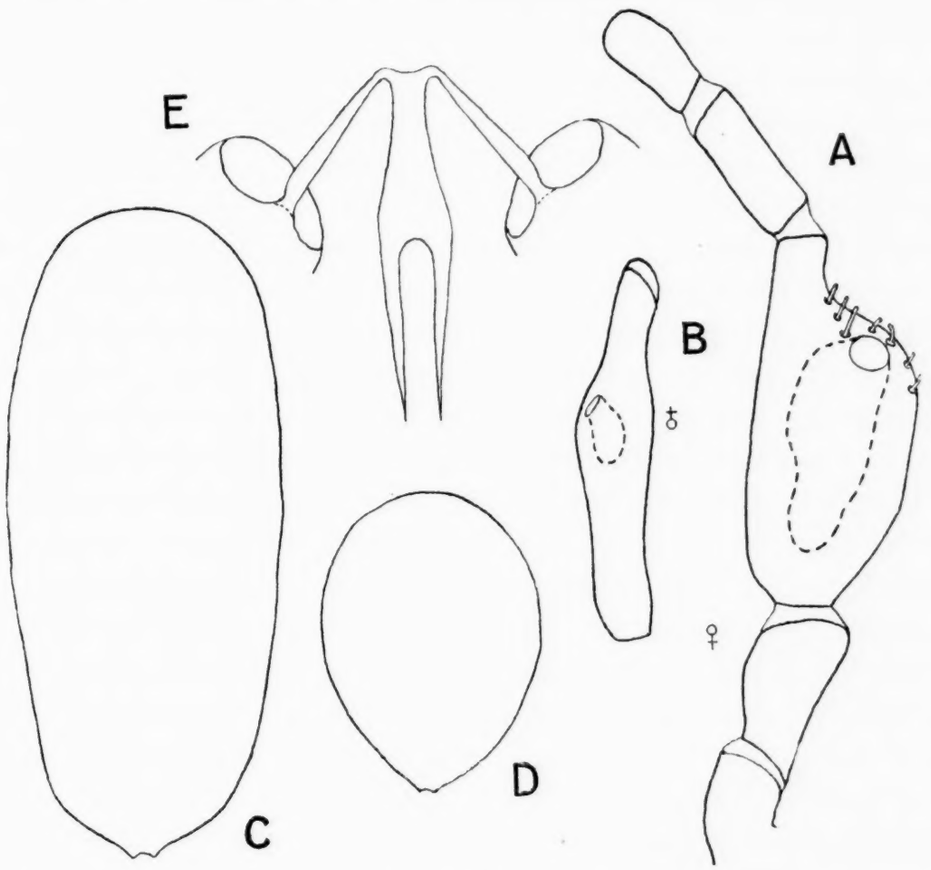

Fig. 10. *Forcipomyia inornatipennis* (Aust.). *A*—Palp of ♀. *B*—Third segment of palp of ♂. *C* and *D*—Spermatheca of ♀ to show variation in size and shape. *E*—Harpes of ♂, ventral view. *A* and *B* × *c.* 265; *C* and *D* × *c.* 400.

of about twelve hairs. The first segment rather large. The basal segments of the flagellum sub-equal, from sub-spherical to oval, the fourth and the tenth in one specimen measuring 12 by 12, and 15 by 9 units respectively. Segments 11 to 15 sub-cylindrical, elongated, 11 to 14 a little over four times as long as broad (about 30 by 7 units), with a distinct ' collar,' the fifteenth rather longer (about 43 by 7 units) and ending in a blunt stylet. The combined length of

segments 11 to 15 greater than that of segments 3 to 10, or 4 to 10, *e.g.* in one specimen measuring 165 units as compared with 113, or 98 units. In the male similar to those of *F. castanea*, but the terminal segments longer, the lengths of the last five segments in one specimen measuring 18, 65, 43, 38, and 44 units respectively. The combined length of segments 12 to 15 greater than that of segments 3 to 11, *e.g.* in one specimen 190 units as compared with 152 units. *Thorax* almost uniformly brown to very dark brown, shoulders rather paler brown; clothed with longish yellow hairs and short brown to yellowish hairs, and bearing also numerous small scales. Pleura a rather lighter brown than the dorsum. Scutellum and post-scutellum dark brown to almost black, the former bearing numerous bristles and hairs which in the female are more abundant than in the male. *Wings* rather densely clothed with dark brown lanceolate hairs, and bearing numerous long, striated scales on the costa and the first and third veins, which are very dark and dense, and give the wing in this region a darker appearance than elsewhere. There is no pale spot in the middle of the wing as in *F. castanea*, but the extreme base is somewhat lighter coloured than the rest. The wing surface is slightly infuscated in the neighbourhood of the radial cells and the adjacent intercalary vein and along the rami of the fourth and fifth veins. First and third veins fused basally, the first radial cell therefore obsolete, but the second well-formed. Costa extending to about the middle of the wing. Bifurcation of the fifth vein a little proximal to the level of the end of the costa in both sexes. In the male the wing is paler, less densely clothed with hairs and scales, longer, and narrower than in the female. Halteres with cream-coloured knobs. *Legs* yellowish-brown, the terminal tarsal segments somewhat infuscated; rather hairy and bearing also numerous scales. The hind legs may be entirely without dark bands, or may have well-defined dark brown bands on each side of the knees as in *F. biannulata*, or on the femora only; the middle legs may also have similar bands, but they are usually less well-developed than on the hind legs. The presence or absence of the bands on the legs appears to depend on the depth of general colouration of the specimen, the darker individuals showing the greater development of the banding on the legs. In the male, the first tarsal segment is about half the length of the second on the fore and hind legs; shorter, about

one-third the length of the second, on the middle legs ; in the female, the first tarsal segment is about two-thirds the length of the second on the fore legs, three-eighths on the middle legs, and a half on the hind legs. Claws and empodium similar to those of *F. castanea*. *Abdomen* uniformly brown to dark brown in the female, densely clothed with brown and golden-brown hairs, and long, striated scales which are particularly dense on the lateral aspects ; in the male paler, the proximal segments pale brown with more or less complete, broad, dark brown bands, and sparsely clothed with long, dark brown and golden-brown hairs, and bearing fewer scales. Hypopygium very dark brown. Cerci of the female paler than the rest of the abdomen. Spermathecae (fig. 10, C and D) two, very highly chitinised, oval, often unequal and rather variable in size, those measured ranging in length from 80μ to 130μ, and in breadth from 60μ to 80μ ; practically no part of the duct is chitinised.

HYPOPYGIUM similar to that of *F. castanea* but more highly chitinised and darker brown. Ninth segment similar to that of *F. castanea*. Forceps similar to those of *F. castanea* but darker ; claspers dark brown. Harpes (fig. 10, E) united by their basal halves, fork-like, the prongs pointed but not filiform. Aedoeagus similar to that of *F. biannulata*.

LARVA (fig. 11). A gravid female taken in the Accra laboratory on the 26th June, 1921, and imprisoned in a tube containing vegetable débris was observed on the following day to have laid eggs. Fifteen days later numerous larvae were found in the tube, but unfortunately none of them pupated, and six days later (18th July) it was found that all were dead. The dead larvae were preserved and are briefly described here. They are small, length about 2 mm., and presumably not fully grown ; dull white in colour, and armed with brownish setae and hairs. *Head* brown, without definite colour pattern. Macrochaetae *p* and *q* long, slender, slightly barbed, tapering to sharp points ; *p* longer than *q*. Antenna terminating in a small spine which, however, is longer than the corresponding spine in *F. castanea*. *Body :* admedian dorsal spines (*a*) small, hastate on segments 3 to 8, but much reduced on the other segments. On the fifth and sixth segments they are largest ; length about 78μ ; length and breadth of the terminal, spear-like portion about 33μ and 22μ respectively, the point not drawn out as in *F. castanea*. Dorso-

lateral setae (*b* and *d*) arising from a large double tubercle, both stout, brown, barbed setae; *d* straight and pointed, *b* longer, curved, and blunter. Macrochaeta *c* similar to *d* but shorter and slightly curved, arising from a conical tubercle. Ventro-lateral hairs (*e* and *f*) long and slender, arising from separate tubercles. Prothoracic pseudopod similar to that of *F. castanea*, but the crowns

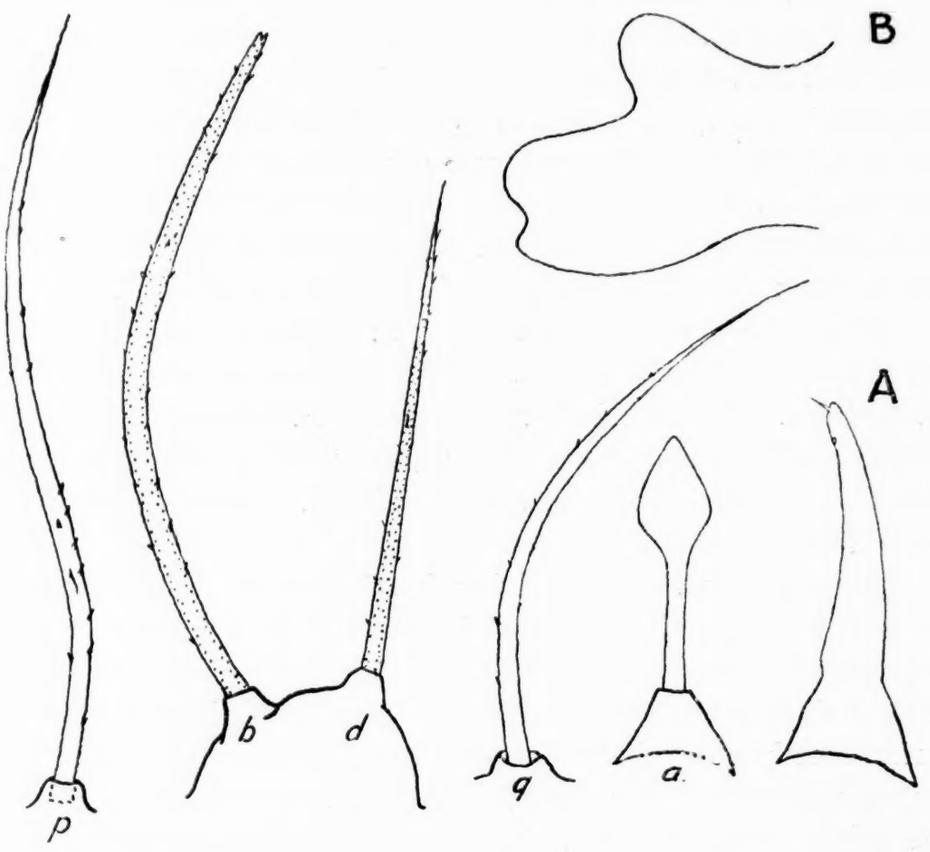

FIG. 11. *Forcipomyia inornatipennis* (Aust.). Larva: *A*—Antenna, *B*—Anal blood-gill of one side, ventral view; *p*, *q*, *a*, *b*, and *d*—macrochaetae lettered according to Saunders. *B* × *c.* 600; rest × *c.* 400.

composed of only five hooks each. Anal blood-gills bilobed. Posterior pseudopod armed with eighteen hooks in two rows, nine on each side, as in *F. castanea*.

GOLD COAST: Accra, numerous specimens, both males and females, taken in the evening on the windows of the laboratory and in bungalows at all times of the year. Obuasi, Ashanti, April, 1920, 1 ♂ (Dr. A. S. Burgess).

NIGERIA: Lagos, June, 1921, 3 ♂♂, 6 ♀♀ (Dr. H. A. Foy); Oshogbo, November 1910, 1 ♂ (Dr. T. F. G. Mayer); Yaba, near Lagos, 20.5.1909, 1 ♂ (Dr. W. M. Graham), 'caught on hand near lamp at 9 p.m.'

TANGANYIKA: Dar-es-Salaam, 16.4.1920, 1 ♀ (W. C. C. Pakes), 's.s. Prinzessin, one mile from shore.'

This species was originally described by Austen (1912) from a single female collected near Lagos. The large number of specimens in our possession have enabled us to describe the male and to add some points of importance with regard to the female. Perhaps our most important observation is that the species is variable and that individuals which are otherwise identical and which have indistinguishable genitalia may have either entirely unbanded legs, banded hind legs only, or banded middle and hind legs. Such characters as the colour markings of the legs without confirmation by morphological differences are, therefore, insufficient for specific distinction (see also *F. castanea*). The forms of *F. inornatipennis* with banded legs can only be regarded as a variety, and as it will be convenient, we propose to refer to them as *F. inornatipennis*, var. *ornaticrus*.

FORCIPOMYIA HIRSUTA, sp.n.

Length of body (one male and two females), 1·6 mm. to 2·2 mm.; length of wing, 1·3 mm. to 1·5 mm.; greatest breadth of wing, 0·4 mm. The body and the wings in the male are slightly longer than in the female. A very hairy, darkish brown species with banded legs.

Head darkish brown, occiput clothed with long, dark brown hairs. Eyes bare; in the female broadly, in the male more narrowly contiguous above. Clypeus, proboscis, and palpi brown; clypeus and palpi bearing rather numerous and long dark brown hairs. Palpi (fig. 12, A) with a rather large first segment; second, fourth and fifth segments sub-equal, about as broad as long, the fourth nearly conical and broadly connected with the fifth; the third segment longer than the fourth and fifth together, inflated basally, with a somewhat variable sensory pit which is sometimes divided into one or two small distal sensory pits in addition to the usual larger proximal one—in the male the sensory pit is reduced. *Antennae:*

In the female first segment rather large, darkish brown, bearing rather numerous, long, stout, dark brown hairs. Torus darkish brown. Segments 3 to 10 quite colourless basally, and dark brown apically, the change of colour taking place abruptly at the level of the whorl of hairs ; segments 11 to 15 entirely dark brown. Segments 3 to 10 bearing long spines, which are almost as long as the segments, twice as stout as the hairs, and with pointed extremities ; and whorls of about fourteen dark brown hairs. Segments 4 to 10 more or less flask-shaped, sub-equal, but gradually becoming a little longer, narrower, and more attenuated distally ; the actual measurements of length and breadth of the fourth and tenth segments in one specimen being 15 by 9 and 19 by 8 units respectively. Segments 11 to 14 sub-cylindrical, tapering a little distally, sub-equal, a little more than three times as long as their basal breadth ; segment 15 rather larger, about five times as long as broad, not tapering distally but ending in a rather long stylet. The combined length of segments 11 to 15 slightly greater than that of segments 4 to 10, but less than that of segments 3 to 10, the actual measurements in one specimen being 129, 124, and 141 units respectively. In the male the first segment a mere ring of chitin ; the torus very large, yellowish-brown ; the flagellum segments pale brown, but the last three darker than the others ; and the plume dark brown basally and pale brown apically. General form of the flagellum segments as usual ; the lengths of segments 11 to 15 in one specimen being 18, 67, 40, 30, and 33 units respectively. The combined length of segments 12 to 15 almost equal to (slightly greater than) that of segments 3 to 11. *Thorax* : Dorsum uniformly darkish brown, glossy, rather sparsely clothed with dark brown hairs, some of which are arranged so as to form a curved row on each side immediately in front of the scutellum ; pleura paler, yellowish brown. Scutellum dark brown at the sides, a little paler brown in the middle ; bearing in both sexes about twenty to twenty-five bristles and hairs, of which ten are especially large. Post-scutellum dark brown, with a small depression posteriorly. *Wings* brownish, darker in the female, without spots, covered with microtrichia as usual, densely clothed with decumbent hairs, and bearing on the costa and the first and third veins numerous dark brown, lanceolate scales ; the hairs are most abundant near the anterior border of the wing and with the dark scales give the appear-

ance of a dark line in this region when examined with a hand lens. Fringe long. Costa reaching to the middle of the wing in both sexes. First and third veins fused basally, but forming distally a cell of some size. Bifurcation of the fifth vein at about the level of the end of the costa in the male, slightly proximal to it in the female. Halteres with buff-coloured knobs. *Legs* yellowish-brown, with darker brown bands at the apex of the hind femora, the base of all the tibiae, and the apex of the fore tibiae and about the middle of the hind tibiae; tarsal segments on all the legs infuscated. The darker markings are less distinct in the male than they are in the female. Legs very

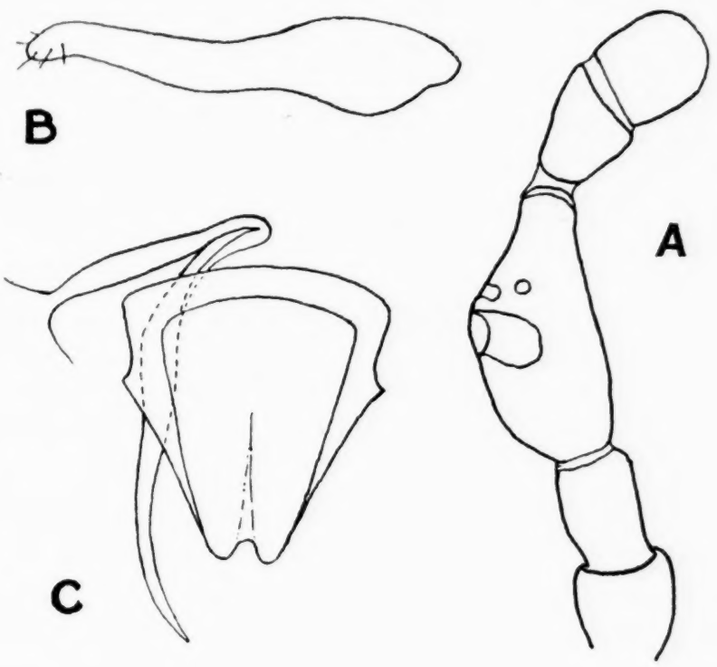

FIG. 12. *Forcipomyia hirsuta*, sp.n. *A*—Palp of ♀. *B*—Clasper of ♂. *C*—Diagram of aedoeagus and one harpe of ♂, ventral view. × *c.* 400.

hairy, the tibiae in particular bearing very long hairs dorsally, some of them almost as long as the segment; bearing also broad scales on the femora and the distal segments. First tarsal segment in both sexes longer than the second on the fore and middle legs, very slightly shorter than the second on the hind legs. Claws and empodium as usual, but the claws in the male do not appear to be bifid. *Abdomen* darkish brown in the female, paler brown in the male and with darker brown markings especially on the posterior segments; in both sexes very hairy posteriorly, and bearing also

numerous scales. Hypopygium of the male brown. Spermathecae two, highly chitinised, sub-equal, pyriform, about 65μ by 45μ, the proximal end tapering to merge with the duct.

HYPOPYGIUM (fig. 12, B and C). Ninth segment of the usual form, hairy, lateral parts of the posterior extension of tergite slightly chitinised; Forceps: side-pieces oval, short and broad, densely clothed with dark brown and yellowish hairs, some of which are extremely long; claspers darkish brown, not expanded at the end. Harpes blade-like, long, narrow plates of delicate chitin, only slightly curved, tapering distally, with pointed extremities, somewhat resembling those of *F. venusta*. Aedoeagus feebly chitinised, but slightly denser at the sides and base than elsewhere, in ventral view triangular, with the apex notched.

GOLD COAST: Accra, 15.7.1920, 1 ♀., and 22.1.1921, 2 ♀♀, taken in the evening on the windows of the laboratory; Nsawam, near Accra, 19.11.1921, 1 ♂ and 1 ♀, reared from decaying banana fibre.

FORCIPOMYIA TIGRIPES, sp.n.

Length of body (one male), 1·8 mm.; length of wing, 1·3 mm.; greatest breadth of wing, 0·3 mm. A light brown species with banded legs.

Head darkish brown. Eyes bare, broadly contiguous above. Clypeus, proboscis and palpi brown. Palpi similar to those of *F. castanea*. *Antennae* pale brown. Proximal segments similar to those of *F. castanea*, but the five distal segments (11 to 15) dissimilar, measuring respectively about 19 by 8, 35 by 7, 29 by 6, 23 by 6, and 31 by 7 units, the last segment without a basal expansion, rather broader than the fourteenth, and ending in a blunt stylet. The combined length of segments 12 to 15 less than that of segments 3 to 11, and a little less than that of segments 4 to 11. *Thorax* rather dark brown dorsally. Pleura pale brown. Scutellum and post-scutellum dark brown, the former bearing about sixteen bristles and a dozen small hairs. *Wings* long and slender, densely clothed with lanceolate hairs and bearing dark brown scales on the costa and the first and third veins. Wings a pale colour, with a small dark brown spot covering the second radial cell. Costa reaching not quite to the middle of the wing, first radial cell obsolete, second

well-formed ; bifurcation of the fifth vein a considerable distance distal to the level of the end of the costa. Halteres with creamy-white knobs. *Legs* light brown with darker brown bands which are most distinct on the hind legs. Clothed with pale brown scales in addition to hairs. Bands on the hind legs covering the apical halves of the femora, the basal sixth and the middle third of the tibiae, the basal two-thirds of the first tarsal segments, and about the

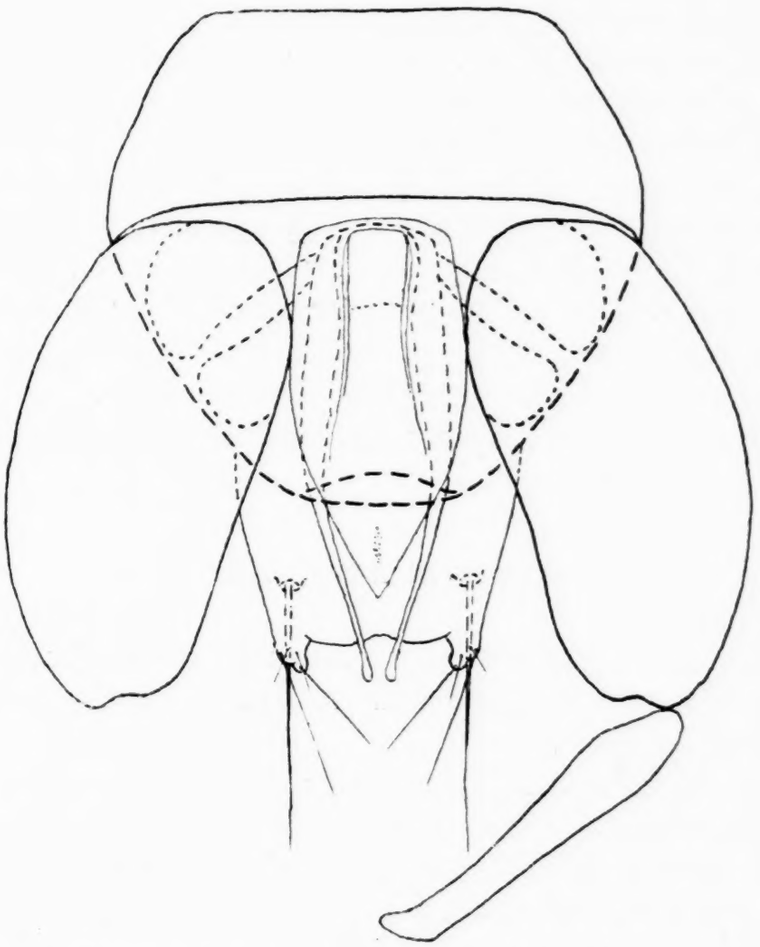

FIG. 13. *Forcipomyia tigripes*, sp.n. ♂ : Diagram of hypopygium. × *c.* 400.

middle two-thirds of the second, third and fourth tarsal segments. The bands on the fore and middle legs much less distinct and smaller, the only conspicuous ones being on each side of the knees, and on the basal two-thirds of the first two tarsal segments. First tarsal segment about equal to the second (44 to 49) on the middle legs, but on the fore legs distinctly longer (45 to 35), and on the hind legs

distinctly shorter (45 to 54) than it. Claws small, not half the length of the fifth tarsal segment, not very strongly curved. Empodium long and hairy. *Abdomen* pale brown, with darker brown bands, often incomplete dorsally, on the middle parts of the segments; clothed with brown scales as well as hairs.

HYPOPYGIUM (fig. 13) dark brown. Ninth segment similar to that of *F. castanea:* sternite moderately hairy; membranous posterior extension of tergite bearing dorsally a pair of large sub-lateral bristles, and furnished with lateral terminal processes each bearing two long and two short hairs. Forceps: side-pieces short and broad (40 by 20 units), dark brown, bearing some very long stout hairs; claspers similar to those of *F. castanea,* not very dark, but infuscated at base and apex. Harpes long and slender, tapering distally but with their ends slightly expanded; they appear to be united across the middle line at the base by a narrow bridge of chitin. Aedoeagus rather long, with a conical posterior termination; mainly membranous, but chitinised laterally, and in ventral view showing on each side a sub-median longitudinal ridge of brown chitin.

GOLD COAST: Accra, 1920, 1 ♂, taken in the evening on a window in the laboratory.

FORCIPOMYIA AETHIOPIAE, sp. n.

Length of body (one male), 1·1 mm.; length of wing, 0·9 mm.; greatest breadth of wing, 0·2 mm. A dark brown midge with hairy eyes; without scales.

Head dark brown. Eyes hairy; the facets rather widely separated, by about 18μ. Clypeus, proboscis, and palpi brown. Clypeus bearing about six hairs. Palpi with first segment small, second and fourth sub-equal, not quite twice as long as broad, fifth small, shorter than the fourth, third about as long as the fourth and fifth together, slightly inflated in the middle, and furnished with a small sensory pit. *Antennae* darkish brown, the torus darker than the flagellum. Third segment smaller than the fourth, with a long (about 30μ) petiole. Segments 4 to 11 of about equal length (10 to 9 units), but rapidly decreasing in breadth from 9 units in the case of the fourth, to 6 units in the case of the eleventh. Segments 12 to 15 elongated, measuring in length and greatest breadth about

27 by 6, 16 by 5, 13 by 5, and 17 by 7 units respectively ; segment 12 shaped like the eleventh, but with the distal end greatly drawn out, segments 13 and 14 sub-cylindrical with a small basal dilatation bearing a whorl of hairs, and segment 15 broader than the rest, tapering slightly distally, and ending in a blunt stylet. The combined length of segments 12 to 15 practically equal to that of segments 4 to 11. *Thorax* uniformly darkish brown dorsally, pleura paler brown. Scutellum dark brown, bearing two lateral and five centro-marginal bristles (two of the latter being small), and about seven

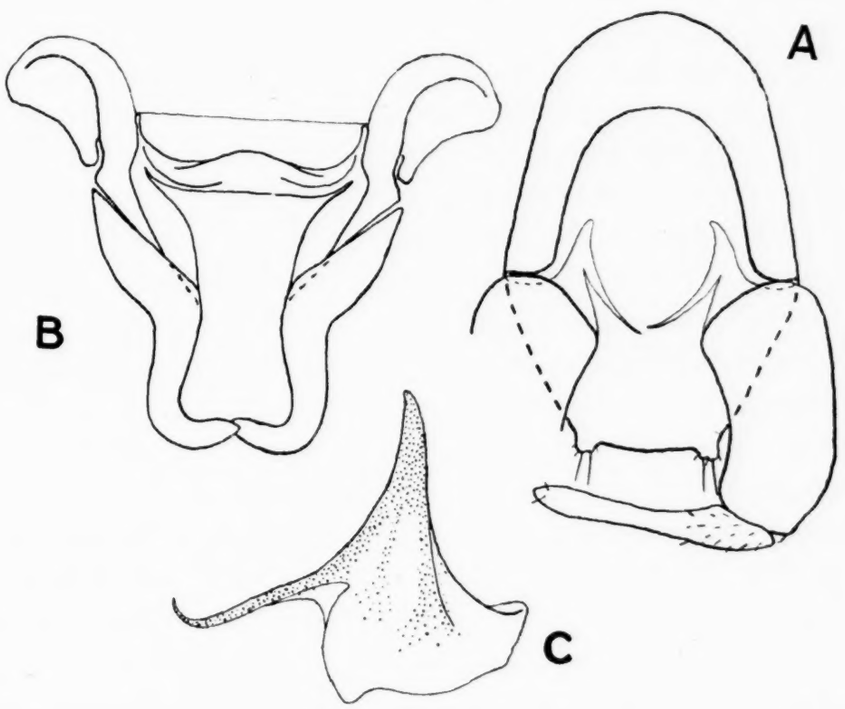

FIG. 14. *Forcipomyia aethiopiae*, sp.n. ♂ : Diagrams of parts of the hypopygium in ventral view : *A*—Ninth segment and forceps, *B*—Aedoeagus, *C*—Harpe. *A* × *c.* 375 ; *B* and *C* × *c.* 900.

smaller hairs Post-scutellum dark brown, darker than the scutellum. *Wings* unadorned, well-clothed with macrotrichia. Microtrichia minute. Frings hairs apparently simple. Costa reaching just beyond the middle of the wing (24 : 46). First radial cell obliterated, second well-formed, but not very long. Halteres with dull white knobs. *Legs* uniformly darkish brown. First tarsal segment more than twice the length of the second on all the legs, about three times the length on the fore and hind legs, rather shorter (36 : 16) on the

middle legs. Claws delicate, slender, more than half the length of the last tarsal segment, with bifid ends. Empodium long and hairy. *Abdomen* darkish brown, sparsely clothed with moderately long hairs.

HYPOPYGIUM (fig. 14). Ninth segment long, dark brown ; tergite densely clothed with long, stout hairs, its posterior margin rounded and bearing at each side a small irregularly shaped process armed with a few short hairs ; sternite very deeply excavated in the middle line posteriorly, the basal strip hairy. Forceps well-developed, side-pieces oval, slightly convex outwards, about twice as long as broad, and well-clothed with long, strong hairs ; claspers highly chitinised, dark brown, the basal third covered by small hairs. Harpes apparently represented by an irregularly triangular plate of chitin on each side with a thin process directed towards the middle line where it is contiguous with, but so far as could be made out not connected with, its fellow of the opposite side. Aedoeagus a very complex structure showing in ventral view a highly chitinised basal bar, and on each side projecting posteriorly a highly chitinised hook-shaped process, the two hooks directed inwards at their ends and sometimes overlapping, but not fused together.

GOLD COAST : Accra, 1920, 1 ♂, taken on a window in the laboratory.

The systematic position of this species, of which we possess only a single male, is somewhat obscure, and it is placed here, with other species belonging to the genus *Forcipomyia*, provisionally only.

FORCIPOMYIA INGRAMI, Cart.

Carter (1919) has described this species fully and has given excellent drawings of the more important parts, including a ventral view of the hypopygium of the male. The description of the latter, however, requires modification. At the same time, as we have at our disposal abundant material (including Carter's co-types), it may be of advantage to give additional or more exact details regarding certain parts which are now necessary for identification of the species.

The species is somewhat variable both in size and colour : usually dark brown as described by Carter, but sometimes much paler. Without scales. Eyes bare, apparently broadly contiguous, but

actually narrowly separated above in both sexes. The twelfth segment of the antenna of the male nearly three times as long as the eleventh and twice as long as the fourteenth, the thirteenth and fifteenth (including the stylet) sub-equal, a little longer than the fourteenth; the actual measurements of the last five segments (11 to 15) in three specimens of average size being approximately 14, 40, 29, 20, and 26 units respectively. The combined length of segments 12 to 15 slightly greater than that of segments 4 to 11, but less than that of segments 3 to 11. In the female occur whorls of about ten hairs and spines on the basal segments twice as stout as the hairs; the combined length of segments 11 to 15 slightly greater than that of segments 3 to 10, namely, about 84 to 75 units.

Scutellum in the male bearing about eight especially large bristles in a transverse row as in the female, but only about fifteen to twenty smaller bristles and hairs, that is about half the number in the female. In the male the costa reaching to the middle of the wing; bifurcation of the fifth vein distal to the level of the end of the costa. Halteres usually with white knobs in both sexes, sometimes with brown knobs, the pigment in them being in both cases white and the chitinous covering more or less infuscated. Fringe of the wing of the usual form, not, as shown in Carter's figures, a single row of hairs. First tarsal segment in the male slightly longer than the second on the fore legs, slightly shorter than the second on the middle and hind legs. Carter's statement that on the middle legs the first tarsal segment is slightly longer than the second is clearly an error, his co-type specimen showing the reverse relationship. Spermathecae (fig. 15, D) of the female, two, sub-equal, usually pyriform, with a terminal sub-spherical portion and a proximal conical portion leading to the duct, rather variable in size, but the greatest breadth usually about 50μ to 60μ and the total length about 65μ to 85μ.

HYPOPYGIUM (fig. 15, A, B and C). Ninth segment darkish brown, large, the proximal end long, very hairy both dorsally and ventrally. Posterior border of the sternite not excavated in the middle. Tergite with a membranous posterior extension which reaches back as far as the ends of the side-pieces, and is strengthened by a narrow rod of chitin on each side, ending in a process bearing three or four hairs—this is the process referred to by Carter as the basal lobe of the side-pieces. Forceps: side-pieces dark brown, not quite twice as

long as broad, almost reniform, with a rounded posterior extremity; claspers pale brown, not highly chitinised, broad with somewhat spoon-like ends. Harpes without posterior rod-like structures, dorsal root-like basal process of the side-pieces long and slender with a small barb at its posterior third, fused proximally with its fellow of the opposite side to form a wide arch, as figured by Carter, in the middle of which is a slight thickening. Aedoeagus delicately chitinised (excepting at the base, where it is stronger) especially laterally, tapering distally so as to appear conical in ventral view, with a small terminal lip, which is slightly everted ventrally.

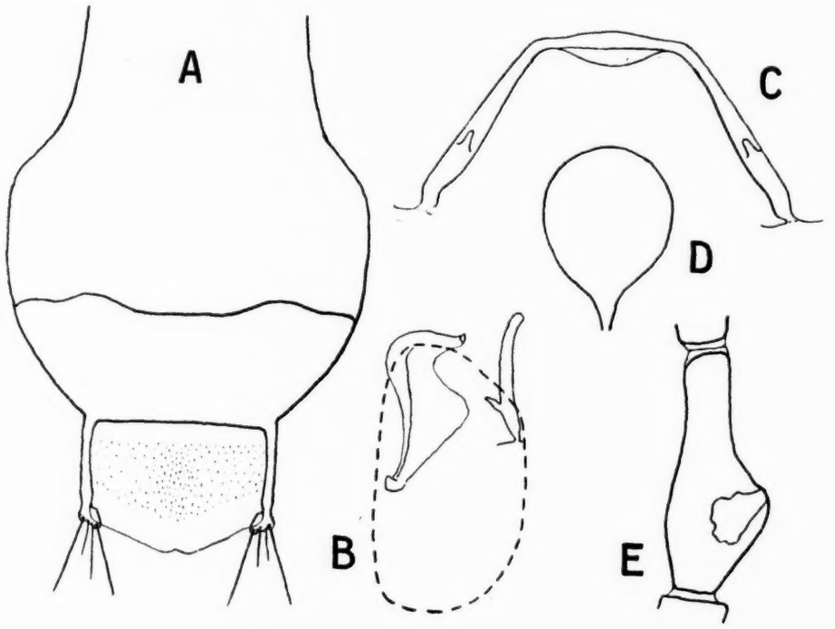

FIG. 15. *Forcipomyia ingrami*, Carter. *A*—Outline of ninth segment after removal of forceps and aedoeagus, ventral view. *B*—Hypopygium of ♂, lateral view, to show the relative positions of the aedoeagus and the dorsal process arising at the base of the side-piece. *C*—Dorsal processes arising from the bases of the side-pieces, ventral view of a disarticulated and slightly flattened specimen. *D*—Spermatheca of ♀. *Forcipomyia nigeriensis*, sp.n. ♀: *E*—Third segment of palp. *A*, *B*, *D*, and *E* × *c.* 265; *C* × *c.* 400).

GOLD COAST: Accra, abundant in the laboratory and in houses at all times of the year; Bole, 3.9.1911, 1 ♂ (Dr. A. Ingram); Kasunya, July, 1922, 1 ♂ (Dr. J. W. S. Macfie), reared from plants of *Pistia stratiotes* from the lagoon.

NIGERIA: Ilorin, 23.4.1912, 1 ♀ (Dr. J. W. S. Macfie); Yaba, near Lagos, 25.5.1909 and 28.6.1909, 2 ♀♀ (Dr. W. M. Graham), also 25.7.1913, 1 ♀ (Dr. J. W. S. Macfie); Calabar, February, 1922,

1 ♂ (Dr. E. C. Braithwaite); Victoria, April, 1921, 1 ♀ (Dr. L. H. Booth).

This species appears to resemble closely *F. rufescens* (Kieffer 1918), a Tunisian species, but to differ from it in general colouring, being usually dark brown with yellowish-brown legs, and not reddish with pale yellow legs. Moreover, *F. rufescens* is described as having the first and third veins of the wing fused in their proximal two-thirds, whereas in *F. ingrami*, Cart. (female) they are contiguous in their basal halves, but actually form a very narrow cell difficult to distinguish, and the scutellum yellow in the female and dark brown in the male, whereas in *F. ingrami* it is brown in both sexes. *F. seychelleana* (K.), *F. seychelleana*, var. *fulvithorax* (K.), and *F. kribiensis* (K.), also closely resemble *F. ingrami*; indeed, in the present state of knowledge it is difficult to select from the descriptions, satisfactory points of distinction, and it is possible some of the names are synonyms. An examination of the genitalia, which in Kieffer's species has not yet been made, would probably help in elucidating this matter.

FORCIPOMYIA EXIGUA, sp.n.

Length of body (two females), 1·0 mm.; length of wing, 0·8 mm.; greatest breadth of wing, 0·3 mm. A small, brown species with a pale brown scutellum, three rather indistinct brown bands on the dorsum of the thorax, and large white halteres.

Head darkish brown, occiput densely clothed with rather long brown hairs. Eyes bare, attenuated and narrowly separated above, the facets about 25μ apart. Clypeus, proboscis, and palpi brown. Palpi (fig. 16, A) with the second segment small, only slightly longer than broad; fourth and fifth sub-equal, about twice as long as broad; third as long as the fourth and fifth together, inflated at the base and furnished with a sensory pit of moderate size. *Antennae* uniformly brown; torus rather darker than the flagellum segments. Segments 3 to 10 bearing stout, colourless spines, about twice as broad as the hairs, but not so long as the segments; and whorls of about twelve brown hairs. Flagellum segments forming an almost continuous series with no abrupt change of shape between the tenth and eleventh segments, but the distal segments rather longer and more attenuated

apically ; the fourth measuring in one specimen 8 by 7, and the fourteenth 11 by 6 units, the fifteenth segment broader than the others, about 14 units long, including the short terminal stylet. The combined length of segments 11 to 15 practically equal to that of segments 4 to 10, namely, about 60 units. *Thorax* brown ; dorsum darker brown than the pleura, with three broad longitudinal darkish brown stripes, the middle one deficient posteriorly and the lateral ones deficient anteriorly. Scutellum pale brown, bearing an irregular transverse row of about ten bristles, and about fourteen smaller hairs. Post-scutellum brown, about the same colour as the mesonotum. *Wings* unadorned, densely clothed with decumbent hairs, but devoid of scales. Microtrichia minute. Fringe as usual. Costa short, not reaching to the middle of the wing. First and third veins contiguous in their basal two-thirds, the first cell obsolete or indistinct, the second definite but small (fig. 16, B). Bifurcation

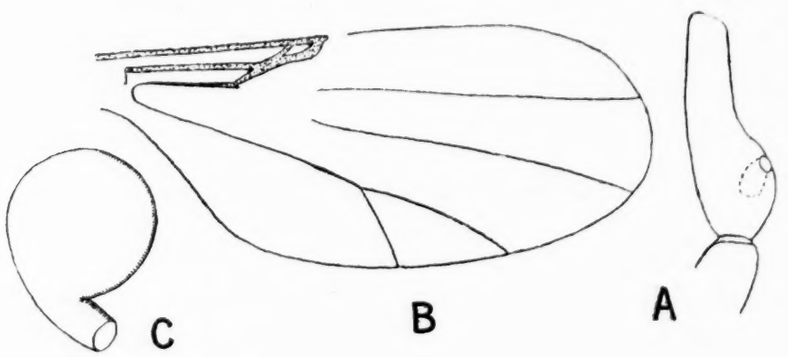

FIG. 16. *Forcipomyia exigua*, sp.n. ♀: *A*—Third segment of palp, *B*—diagram of wing venation, *C*—Spermatheca. *A* and *C* × *c.* 400, *B* × *c.* 80.

of the fifth vein a little distal to the level of the end of the costa. Halteres relatively large, with white knobs. *Legs* uniformly pale brown, bearing shortish hairs, and devoid of scales. First tarsal segment about two-and-a-half times the length of the second on all the legs ; on the hind legs about as long as the four succeeding segments. Claws and empodium as usual. *Abdomen* brown, tergites with broad darkish brown bands covering almost the entire surface ; sparsely clothed with hairs, but devoid of scales. Spermatheca (fig. 16, C) single, highly chitinised, shaped like a retort, the body sub-spherical, about 45μ in diameter, the chitinised portion leading to the commencement of the duct unusually broad, about 12μ.

GOLD COAST: Accra, 21.1.1920, 2 ♀♀, and 4.2.1922, 1 ♀, taken in the evening upon the windows of the laboratory.

This species differs from all the other species of the genus which we have examined in having only a single spermatheca.

FORCIPOMYIA ASHANTII, sp.n.

Length of body (one male), 1·6 mm.; length of wing, 1·2 mm.; greatest breadth of wing, 0·35 mm. A rather light brown species; without scales.

Head darkish brown. Eyes bare, the facets separated dorsally by a narrow line. Clypeus, proboscis, and palpi darkish brown. Palpi similar to those of *F castanea*. *Antennae* rather pale brown, the plumes dark brown with paler brown tips. Torus very large, dark brown, without hairs. Flagellum segments similar in general characters to those of *F. castanea*, but the last three rather shorter; the lengths of the five terminal segments in one male were 13, 52, 23, 17, and 25 units respectively. The combined length of segments 12 to 15 about equal to that of segments 3 to 11. *Thorax*: dorsum uniformly light chestnut-brown with a slight greyish pruinescence, hairs scanty and short. Pleura much the same colour as the dorsum, not distinctly paler as in *F. castanea*. Scutellum about the same colour as the scutum, bearing fewer setae and hairs than in *F. castanea*, namely about 30. Post-scutellum slightly darker brown than the scutellum. *Wings* in some lights white and finely iridescent; with a distinct pale golden spot about the middle of the anterior border covering the distal radial cell. On each side of this pale spot is a patch of dark brown macrotrichia, the distal patch being especially large and distinct, and half-way between this dark patch and the tip of the wing, the wing surface is slightly infuscated. In addition to these markings there are a number of dark brown macrotrichia in the region of the bifurcation of the fifth vein, which stand out rather prominently. Macrotrichia rather scanty, mostly pale brown. Costa reaching to the middle of the wing, first and third veins fused in their basal halves, but forming a rather large distal cell, bifurcation of the fifth vein slightly distal to the level of the end of the costa. Halteres very pale brown, the knobs containing white pigment. *Legs* almost uniformly yellowish-brown,

but with a rather inconspicuous, slightly darker brown spot at the distal end of the hind femora, and, in one specimen, with a trace of a brown band on the hind tibiae a little below the knee. First tarsal segment of the fore legs about the same length as the second; those of the middle and hind legs shorter than the second, about three-quarters the length. Claws and empodium as in *F. castanea*. *Abdomen* pale brown, with darker brown bands on the segments which are broadest and most conspicuous on the ventral surface and on the posterior segments. Hypopygium brown, the side-pieces pale brown apically and darker brown basally.

HYPOPYGIUM (fig. 17). Ninth segment similar to that of *F. castanea*, but sternite notched in the middle line posteriorly, and the hairs on the sternite fewer, reduced in the middle line to a single

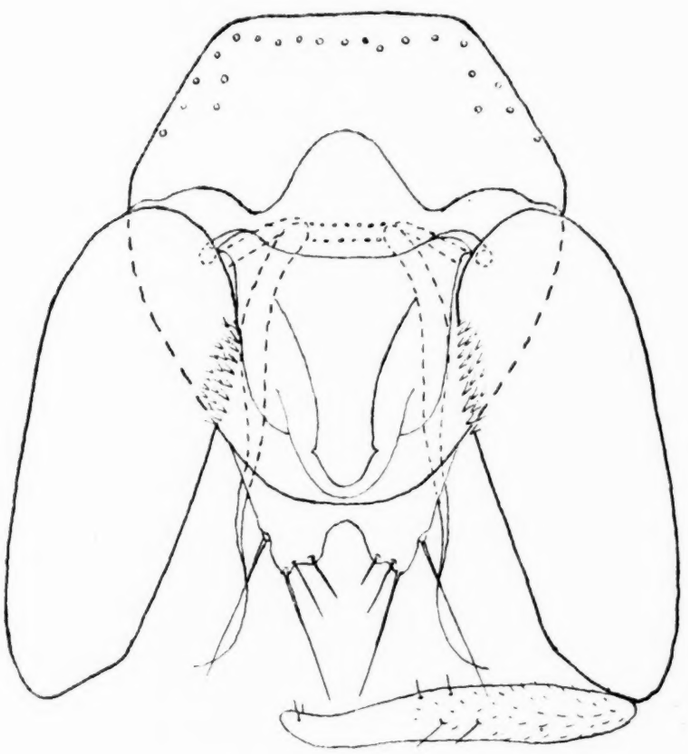

FIG. 17. *Forcipomyia ashantii*, sp.n. ♂: diagram of hypopygium, ventral view. × *c.* 400.

basal row. Forceps generally similar to those of *F. castanea*, but side-pieces rather long, and bearing on the inner basal aspect a patch of short, stout spines or teeth. Harpes united basally by a narrow

band of chitin, ending in long, slender filaments, resembling thus those of *F. biannulata*. Aedoeagus bluntly conical, rather long, not unlike that of *F. tigripes*.

GOLD COAST: Obuasi, Ashanti, April, 1920, 3 ♂♂ (Dr. A. S. Burgess); Nsawam, near Accra, 1 ♂, reared from vegetable matter taken from a rot-hole in a tree; Accra, 2 ♂♂, taken in the evening upon windows in the laboratory.

NIGERIA: Yaba, near Lagos, 23.5.1909, 1 ♂ (Dr. W. M. Graham), 'in bedroom at 9 p.m.'

This insect resembles in many respects *F. castanea*, but is a paler, more uniform, brown colour, the thorax not notably darker dorsally than laterally, and the hind femora with only an inconspicuous darker spot. The genitalia of the male are quite distinct from those of *F. castanea*.

FORCIPOMYIA MELANCHROA, sp.n.

Length of body (one female), 3·0 mm.; length of wing, 1·6 mm.; greatest breadth of wing, 0·6 mm. A large, very dark brown, almost black, species; without scales.

Head very dark brown. Eyes bare; broadly contiguous above, the facets separated by a narrow line. Clypeus, proboscis, and palpi dark brown. Palpi similar to those of *F. castanea*: second, fourth, and fifth segments sub-equal, a little longer than broad; third about as long as the fourth and fifth together, its distal half narrow, its basal half greatly inflated and furnished with a large sensory pit. *Antennae* uniformly dark brown; torus rather small, scarcely darker than the flagellum segments. The large spines on segments 3 to 10 colourless or very pale brown, straight, pointed, as long as the segments, and at least twice as stout as the hairs; whorls of about twenty short, dark brown hairs. Segments 4 to 10 oval, sub-equal, the length and greatest breadth of the fourth and tenth being about 18 by 14, and 16 by 12 units respectively. Segments 11 to 15 elongated, sub-cylindrical; 11 to 14 sub-equal, about one-third longer than the tenth, and from two to three times as long as their basal breadth; the fifteenth slightly longer, about four times as long as broad, ending in a small blunt stylet. The combined length of segments 11 to 15 equal to that of segments 4 to 10, namely about

122 units. *Thorax* very dark brown, almost black. Dorsum showing indistinctly (because the insect is so dark), the three broad longitudinal bands common in this genus, and also a small short lateral band on each side. Pleura dark brown. Scutellum and post-scutellum almost black; the former bearing numerous bristles and hairs. *Wings* brownish, unadorned, darkest about the middle near the anterior border, densely clothed with dark brown decumbent hairs, but devoid of scales. Costa reaching a little beyond the middle of the wing. First and third veins darkish brown, fused in their proximal halves, but forming a distinct distal cell. Cross-vein and petiole of the fourth vein about equal. Bifurcation of the fifth vein just proximal to the level of the end of the costa. Halteres with pale brown knobs. *Legs* uniformly very dark brown, densely clothed with shortish, very dark brown hairs, devoid of scales. First tarsal segment about one-quarter longer than the second on the fore legs, and practically equal to the second on the middle and hind legs. Claws and empodium as usual. *Abdomen* uniformly very dark brown, well-clothed with shortish, very dark brown hairs, but devoid of scales. Spermathecae two, highly chitinised, oval, large, unequal or sub-equal, about 200μ to 300μ by 120μ to 150μ; practically no part of the duct chitinised.

GOLD COAST: Accra, 26.12.1920, 1 ♀, taken in the evening in a bungalow; Obuasi, 23.11.1906, and 5.7.1907, 2 ♀♀ (Dr. W. M. Graham).

FORCIPOMYIA NIGERIENSIS, sp.n.

Length of body (one female), 2·3 mm.; length of wing, 1·5 mm.; greatest breadth of wing, 0·5 mm. A large, dull brown species with some general resemblance to *F. inornatipennis* (Aust.), but with wings not so hairy, and legs a dull brown in place of a yellow brown. General colouring similar to that of *F. ingrami*. Without scales.

Head darkish brown. Eyes bare, contiguous above but rather narrowly, the facets separated by a narrow line. Clypeus, proboscis, and palpi darkish brown. Palpi (fig. 15, E) with second, fourth, and fifth segments sub-equal, not twice as long as broad; third segment as long as the fourth and fifth together, ham-shaped, the basal half much inflated and furnished with a large sensory pit which

has a small, circular, anterior opening. *Antennae* uniformly darkish brown. Torus about the same colour as the flagellum segments, rather small, bearing about a dozen hairs. Segments 3 to 10 bearing large spines, about twice as broad as the hairs, not quite as long as the segments, and with rather blunt ends ; and whorls of about twelve dark brown hairs which are mostly only slightly longer than the segments. Flagellum segments forming an almost continuous series with no abrupt change of shape between the tenth and the eleventh segments, but the terminal segments rather longer and more constricted apically, the fourth measuring 16 by 12, and the fourteenth 19 by 10 units. Last segment unfortunately missing. So far as can be judged, however, the combined length of segments 11 to 15 would be rather less than that of segments 4 to 10, or 3 to 10, namely, about 94 as compared with 108 or 126 units respectively. *Thorax* uniformly darkish brown, clothed with short, slender, brown hairs ; pleura not quite so deep a brown. Scutellum and post-scutellum darkish brown, the former bearing about twenty bristles and a few small hairs. *Wings* light brown, unadorned, well-clothed with rather slender decumbent hairs, but apparently devoid of scales. Costa extending slightly beyond the middle of the wing (46 : 81 units). First radial cell obsolete, second well-formed. Bifurcation of the fifth vein a little proximal to the level of the end of the costa. Halteres pale brown. *Legs* almost uniformly darkish brown, hairs rather short, devoid of scales. First and second tarsal segments sub-equal on all the legs, first actually very slightly longer. Claws and empodium similar to those of *F. castanea*. *Abdomen* brown, venter slightly paler than the dorsum, clothed with shortish hairs. Spermathecae two, not very highly chitinised, oval, sub-equal, large, about 150μ by 100μ ; the commencement of the duct chitinised for only a very short distance (about 7μ).

NIGERIA : Lagos, 2.7.1921, 1 ♀ (Dr. H. A. Foy.)

CHANGES IN THE BLOOD IN PRIMARY MALARIA

BY

R. M. GORDON

(*From the Sir Alfred Lewis Jones Research Laboratory, Freetown, Sierra Leone*)

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The observations that follow were made in England on three patients suffering from general paralysis of the insane, all of whom were undergoing treatment by a first attack of malaria, induced as the result of the bites of anophelines infected with *Plasmodium vivax ;* in these cases, therefore, it was possible to observe the blood changes that occurred in a patient suffering from an undoubted primary attack of malaria, the date of acquirement of which was known. So far as the writer is aware, all previously recorded examinations have reference to patients who may—or may not—have been suffering from a primary attack and whose exact date of infection was not known. Ross (1924) has drawn attention to the importance of such observations in cases of primary malaria ; the present work had already been started when his letter was published, but an attempt has been made to investigate one of the problems suggested by him ; that is to say, whether or not there is any noticeable reduction in the total number of red cells before the appearance of parasites in the peripheral blood.

The following were investigated :—

(1) *The total number of red cells per cubic millimetre of blood.* The counts were made in the usual manner with a Thoma-Zeiss haemocytometer, the second drop of blood from the finger being always examined ; it will be noted that the results are expressed in the table to the nearest half-million ; this is due to the fact that a previous series of counts on a normal individual appeared to show that half-a-million was the usual limit of working error.

(2) *The colour index.* A measured volume of the patients' blood was haemolysed in distilled water and compared with the same

volume of a normal individual's blood also in distilled water (the same control being used throughout) ; the percentage of haemoglobin was then compared with the red cell count taken at the same hour, and the result expressed as the colour index.

(3) *Ratio of infected to non-infected red cells.* An adjustable counting eye-piece was used and set to cover a field of forty erythrocytes ; one thousand two hundred and fifty such fields were examined and the number of infected red cells in each field noted. It was found that in an evenly-spread film the number of red cells in such relatively small fields remained very constant, as was shown by counting the actual number of red cells present in every thirtieth field and if necessary, re-adjusting the eye-piece.

As already stated, previous writers' figures are concerned with the blood changes occurring in patients whose incubation period is unknown and whose previous history as regards malaria is uncertain ; it seems unprofitable, therefore, to compare these figures with those considered in the present paper. It may, however, be mentioned that Mannaberg (1894) quotes a large number of figures, both from his own observations and from those of other writers, which show that the reduction in the red cells varies greatly in different cases, but that the haemoglobin reduction is almost always directly proportional to the fall in the erythrocyte count ; that is to say, it is of the simple anaemia type. More comparable figures may be had from animal experiments ; thus Ben-Harel (1923) infected twenty-three birds by intramuscular injection of *Proteosoma praecox ;* he divides the resultant malaria into three types, primary acute infections, extended irregular infections, benign infections. Amongst other observations he clearly demonstrates, by means of daily blood counts, that : (1) In all three types a definite reduction in the total number of red cells occurred during the incubation period ; (2) there was no further fall in the number of red cells after the disappearance of parasites from the circulation ; (3) in all cases in which the infection did not prove fatal, the parasites increased to a certain maximum concentration and then proceeded to fall in numbers at approximately the same rate as that at which they had previously increased. This fall was sometimes interrupted by a relapse, causing a sharp temporary rise.

As the present writer only observed three cases, it would be

Case 1. Male. Aged 38.

Day of observation— 1 = day of infection. 2 = day after infection, etc.	1	4	7	9	12	13	14	16	18	20	22	23	24	26	28	30	32	34	36	38	51
Total number of red cells per c.mm., in millions, to the nearest half-million	5½	5½	5½	5½	5½	...	5½	5½	5	4½	4½	...	3½	4	4	4	4	4	4	4½	4½
Total number infected amongst 50,000 red cells examined	0	0	0	0	0	0	7	62	217	172	180	...	12	0	0	0	0	0	0	0	0
Colour index	1	1	1	1	1	...	1	1	0.9	0.9	0.9	...	1	1	1	1	0.9	0.9	...	0.9	1
Quinine	0	0	0	0	0	0	0	0	0	0	0	30 grs.	30 grs.	0	0	0	0	0	0	0	0

Case 2. Female. Aged 13.

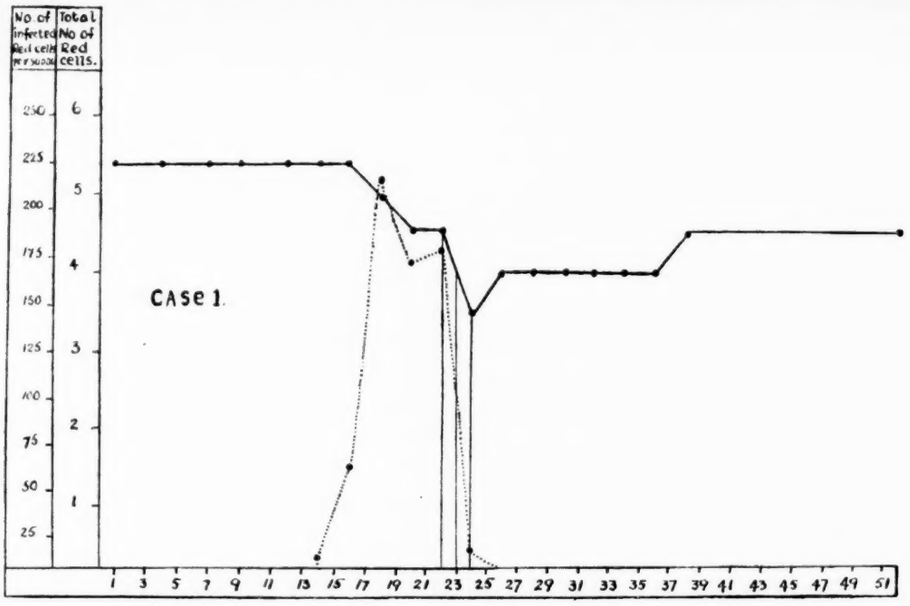
Day of observation— 1 = day of infection. 2 = day after infection, etc.	1	4	7	9	12	15	17	19	21	23	25	27	29	31	33	35	37	48	56
Total number of red cells per c.mm., in millions, to the nearest half-million	5½	5½	5½	5½	5	4½	4½	4½	4½	4	3½	3½	3½	3	3	3	2½	4½	4½
Total number infected amongst 50,000 red cells examined ...	0	0	0	0	0	0	8	80	146	94	33	24	8	25	14	7	0	0	0
Colour index	0.9	0.9	0.9	0.95	0.9	0.9	0.9	0.9	0.9	0.9	0.85	0.85	0.8	0.8	0.8	0.8	0.9	0.85	0.9
Quinine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Quinine grs. 10 daily, from the 35th to the 50th day, inclusive.			0

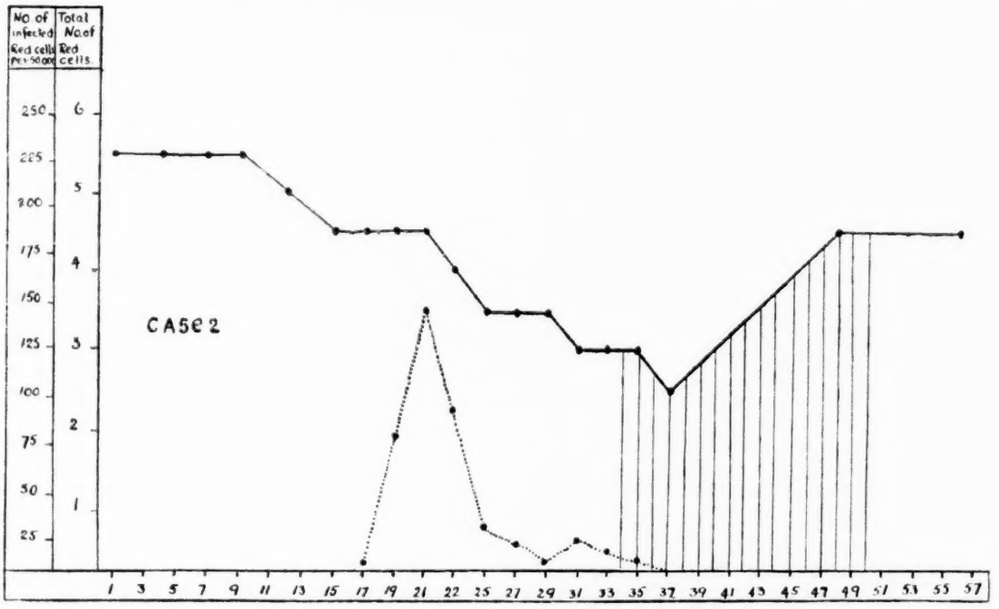
Case 3. Male. Aged 44.

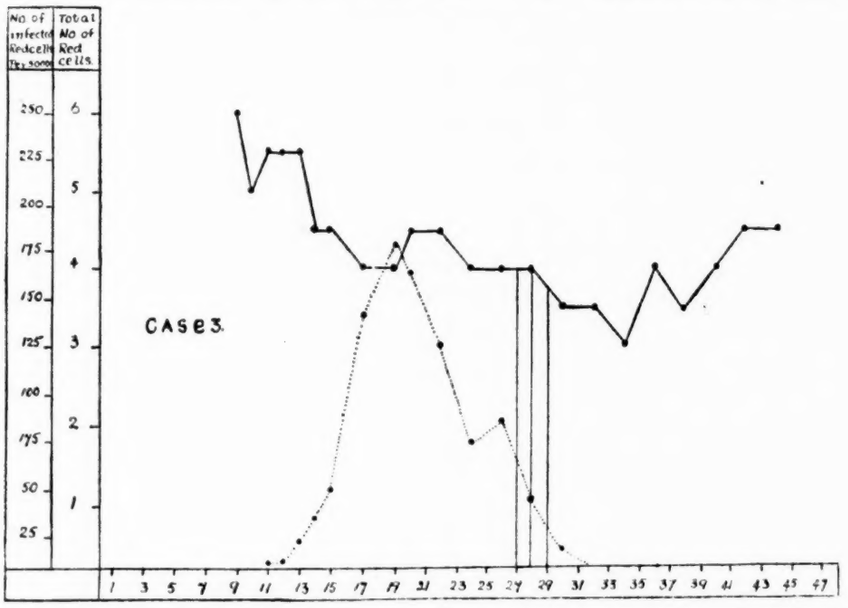
Day of observation— 1 = day of infection. 2 = day after infection, etc.	9	10	11	12	13	14	15	17	19	20	22	24	26	28	29	30	32	34	36	38	40	42	44
Total number of red cells per c.mm., in millions, to the nearest half-million...	6	5	5½	5½	5½	4½	4½	4	4	4½	4½	4	4	4	...	3½	3½	3	4	3½	4	4½	4½
Total number infected amongst 50,000 red cells examined	0	0	2	4	23	30	51	143	178	165	125	75	85	43	...	14	0	0	0	0	0	0	0
Colour index	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Quinine	0	0	0	0	0	0	0	0	0	0	0	0	0	30 grs.	30 grs.	0	0	0	0	0	0	0	0

TABLE II

Graphical records showing the results of blood examinations in three cases of primary malaria.







Explanation of Table. The dotted line represents the total number of infected red cells amongst fifty thousand examined. The continuous horizontal line represents the total number of red cells expressed in millions to the nearest half million. The vertical lines delimit days on which quinine was given, in cases one and three the amount given was thirty grains daily, in case two, ten grains daily. The base line shows the days on which observations were recorded : 1 being the day of infection, 2 the day after infection, etc.

unwise to draw any definite conclusions, but the following points appear to be of interest, especially when compared with Ben-Harel's corresponding figures for birds. (1) In case No. 2 there was a marked diminution in the number of red cells for several days previous to the first appearance of parasites, and the same, though in a less marked degree, is true of No. 3 case; on this particular point, therefore, these two cases accord with Ben-Harel's findings. (2) In at least one patient, the red cell count continued to fall after the disappearance of parasites from the peripheral circulation. This is contrary to all Ben-Harel's findings. (3) The parasites, after reaching a maximum concentration, on or about the fifth day after their first appearance, tended to diminish in numbers; this diminution was slower than the previous increase and took place whether quinine was given or not; it was clearly observable in all three cases, but most marked in cases two and three, in which the maximum concentration was in each case reached somewhere between fourteen and twenty thousand parasites to the cubic millimetre. On the whole, these figures correspond closely with those given by Ben-Harel under the title 'Acute primary infection,' although in some of his cases the parasitic concentration reached was far higher. (4) In two cases where the colour index was estimated it was found to be of the simple anaemia type.

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NOTES ON SOME NEMATODES IN THE MUSEUM OF THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

BY

E. L. TAYLOR, M.R.C.V.S.

(*Received for publication* 10 *November*, 1924)

PORROCAECUM CROCODILI, n sp.

Host :—Crocodile. Position :—Stomach. Locality :—Gold Coast.

This worm is of medium size (6·7 by 0·25 mm. to 32 by 1 mm.). The body tapers gradually towards the head, and terminates abruptly at the posterior extremity ; it is marked by fine transverse striations and a few scattered, minute papillae ; at a distance of about 0·7 mm. from the anterior extremity are two small cervical papillae, placed laterally and just posterior to the nerve ring. The excretory pore is large and somewhat resembles a papilla ; it is, as is usual in this genus, situated between the bases of the two subventral lips. The three lips forming the head (figs. 1 and 2) are 0·13 to 0·18 mm. in diameter, and a little more than half that height ; they present a semi-lunar outline when viewed laterally, and two antero-internal lobules are seen standing above the semicircular anterior margin of the lips ; each of these lobules carries eight or ten denticulations, the rest of the lips is not armed in this way. The dorsal lip carries two long papillae, notched about their middle, while the two subventral lips only have one papilla each, carried towards the ventral side of the centre of the lips. Viewed anteriorly, the lips are seen to have a notch about the middle of their internal aspect, which might be said to divide the anterior lobe of the lip into two. Each lip is marked off from the body by a definite line round its base. The oesophagus is long and slender, measuring up to 4·87 mm. in length (about one-seventh of the total length of the worm), its diameter is uniform throughout the whole length ; a straight, oblong, oeso-

phageal ventriculus is present, measuring about 0·675 mm. long, and there is a well-developed intestinal caecum, extending forward from the end of the intestine to a point slightly anterior to the middle of the oesophagus.

The male measures 8·00 to 24·75 mm. in length and 0·45 to 0·80 mm. in its greatest diameter. The two spicules are of equal

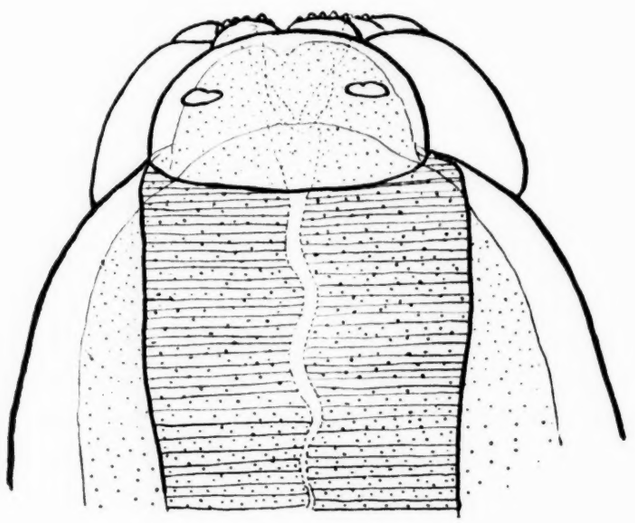

FIG. 1. *Porrocaecum crocodili*, n.sp. Head, dorsal view. × 253.

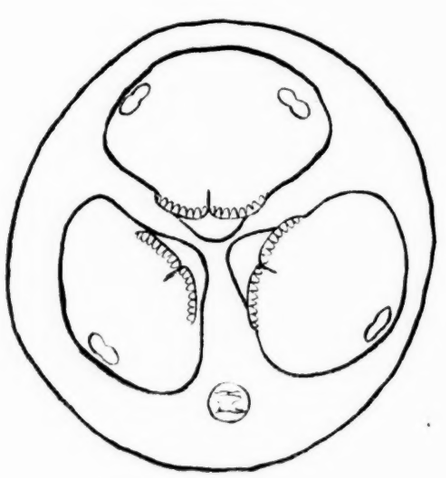

FIG. 2. *Porrocaecum crocodili*, n.sp. Head, anterior view. × 190.

length, tubular in form, not alate, and measuring 0·58 to 0·78 mm. in length. A small but well-defined gubernaculum is present, 0·075 to 0·08 mm. long ; caudal alae are absent. The caudal papillae are in two groups (figs. 3 and 4) : firstly a long file of 30 to 37 pedunculated

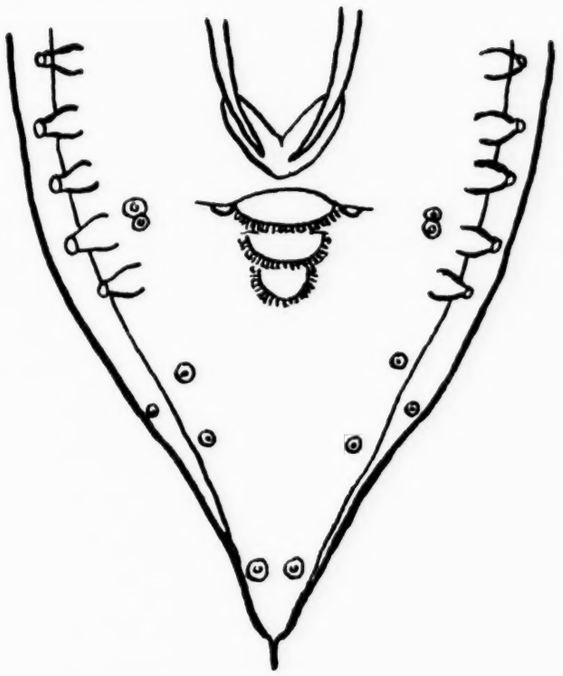

FIG. 3. *Porrocaecum crocodili*, n.sp. Caudal extremity of male, ventral view. × 160.

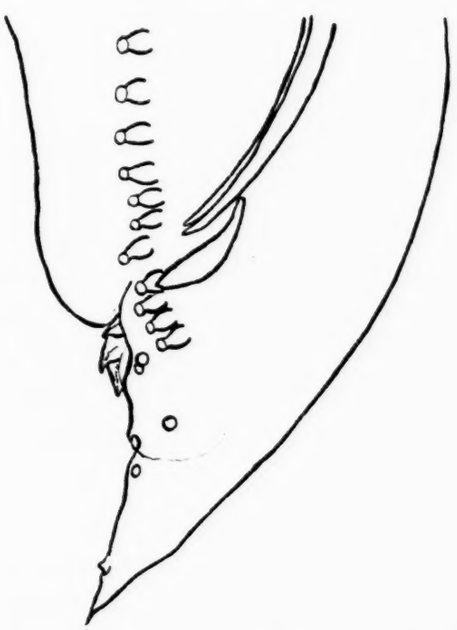

FIG. 4. *Porrocaecum crocodili*, n.sp. Caudal extremity of male, lateral view. × 260.

papillae on either ventro-lateral aspect, the posterior three or four pairs being postanal; and secondly, six pairs of sessile papillae: one ventral, level with the cloaca, a double papilla subventral at the same level, a group of three subventral, forming a triangle half-way between the cloaca and extremity of the tail, and one larger pair placed ventrally further back. Immediately behind the cloaca are three curious semicircular projections from the cuticle; these are directed backwards and have very thin, serrated, free edges; viewed laterally, they look like sharp spines directed backwards from the cloaca. The extremity of the conical tail is an extremely acute point; the distance from the tip to the cloaca is about 0·18 mm.

The female measures 6·7 to 32 mm. in length and 0·25 to 1 mm. in greatest diameter; the vulva is placed just in front of the middle of the body; from here the vagina passes backwards to where it finally divides into the two branches of the uterus. Behind the anus the tail rapidly tapers off to end in a short, sharp point; the length of the extremity beyond the anus is about 0·21 mm.

It must be noted that the specimens available were not gravid.

AMPLICAECUM AFRICANUM, n.sp.

Host :—*Bufo regularis*. Position :—Stomach. Also found in the stomach of a crocodile along with the bones of a frog or toad. Locality :—West Coast of Africa.

This worm is of medium size, measuring 22 to 50 mm. in length and up to as much as 1·2 mm. in thickness. The body gradually tapers towards the head, but terminates abruptly behind in an obtuse cone, which, in the female, is remarkably short. The cuticle is thick and marked with fine transverse striations; the posterior extremity is usually coiled. The excretory pore is situated at about 0·75 mm. from the head, while at a slightly higher level is the nerve ring and two small, sessile cervical papillae. A dorsal papilla may be present, situated about 1·5 mm. from the head. The three lips (fig. 5) are more or less rectangular and measure about 0·27 mm. in diameter; each presents two grooves on its inner aspect, one parallel to the long axis of the body, and one transversely; denticulations are easily seen, and follow the margins of the lips throughout their whole extent. The dorsal lip carries two large papillae, and each

ventro-lateral lip one large and one small papilla ; the large papilla, as usual, being placed towards the ventral side of the lip, and the small one laterally and a little more anteriorly. The large papillae present the peculiar appearance of having a segment cut out of their

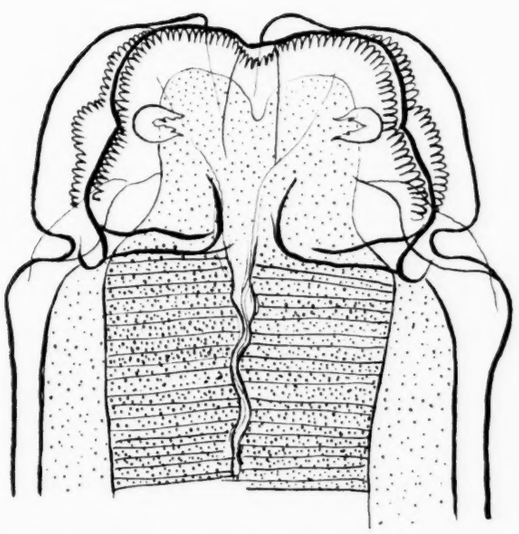

FIG. 5. *Amplicaecum africanum*, n.sp. Head, dorsal view. × 190.

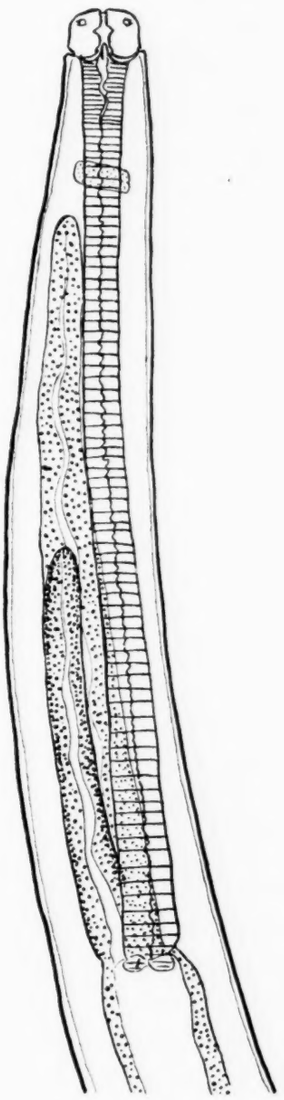

FIG. 6. *Amplicaecum africanum*, n.sp. Oesophageal portion, showing two intestinal caeca. × 40.

inner aspect. The pulp is divided into two equal lobes with even, rounded ends ; small interlabia are present, from which well-marked grooves encircle the bases of the lips. The oesophagus is 3·0 to 4·5 mm. in length (about one-eleventh of the length of the worm)

and about 0·27 mm. wide, throughout its whole length, excepting for a little widening at its two extremities. One large intestinal caecum is present—length about 3·57 mm.—and just where it leaves the intestine it measures about 0·42 mm. in diameter, becoming narrower towards the anterior extremity, and terminating about 0·9 mm. from the head. Sometimes a second caecum is found to be present, and when this occurs, it is only half the length of the one normally seen (fig. 6).

The male measures from 22 to 30 mm. in length and 0·46 to 0·68 mm. in its greatest width; the width at the anus is about 0·35 mm. and the length of the caudal extremity behind the anus about 0·31 mm. Well-developed caudal alae are present, into the

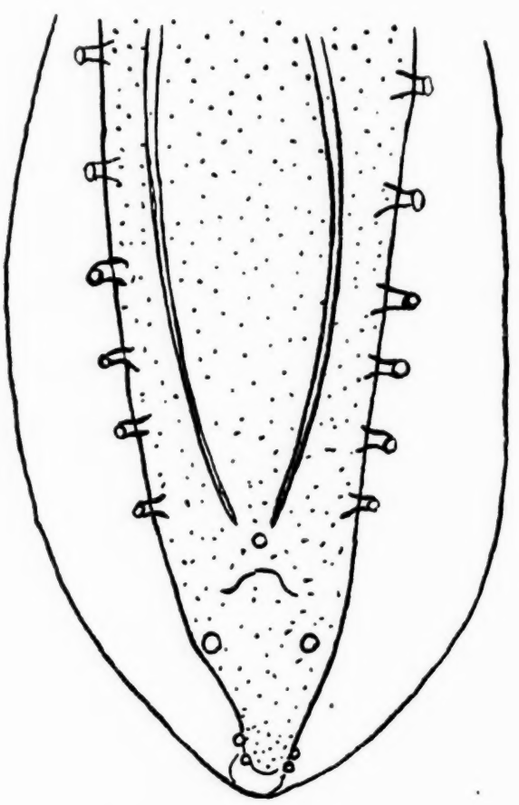

FIG. 7. *Amplicaecum africanum*, n.sp. Caudal extremity of male, ventral view. × 110.

substance of which the pedunculated preanal papillae run (figs. 7 and 8); these papillae are 10 to 12 in number, the anterior members being sessile and small. Postanal papillae are three in number on either side, two very small pedunculated papillae placed laterally

near the extremity, and one larger, sessile papilla placed sub-ventrally just behind the cloaca; there is one unpaired median papilla on the elevation in front of the anus. The spicules are equal and measure about 0·85 mm. in length and 0·013 mm. in diameter; they have a sharp barb at their extremities. Gubernaculum is absent.

The female measures 32 to 50 mm. in length and 0·66 and 1·2 mm. in width; the vulva is situated anterior to the middle, the vagina can be traced backwards from here, and the coils of the ovaries are placed in the posterior part of the body up to 1·5 to 2 mm. from the caudal extremity. The caudal extremity is blunt, extending for about 0·3 mm. behind the anus; it carries a small pointed button at

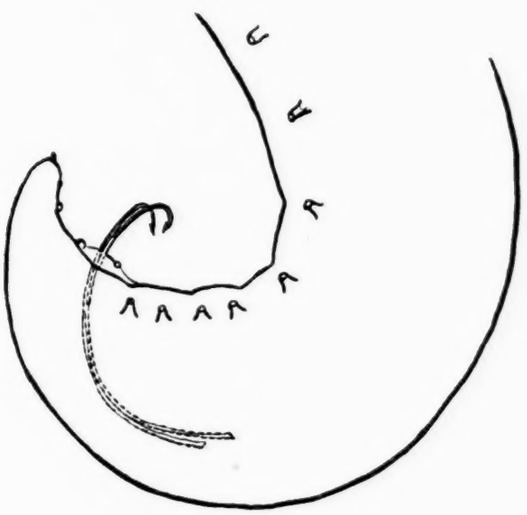

FIG. 8. *Amplicaecum africanum*, n.sp. Caudal extremity of male, lateral view. × 55.

the tip; the width at the anus is 0·63 mm. The eggs are oval and large, 135 by 83μ; the shell is thin and opaque, being marked with numerous small depressions on its surface.

This worm shows considerable similarity with *Amplicaecum colurum* from the eagle (Baylis, 1919), but in the latter species the oesophagus is about one-seventh the complete length of the worm, while the papillae on the dorsal lips are absent. Unfortunately, Baylis had only two female specimens, so that it is impossible to say how closely the species here described resembles *A. colurum:* the identity of the species does not, however, seem probable in view of their two widely different hosts.

On comparison with *Amplicaecum varani*, the only other known species of this genus, the differences are again found to be slight, but, apart from mere difference in size, the intestinal caecum measures one-thirteenth of the body length in the species here described, against only one-twenty-fourth in *A. varani*; and the caudal papillae of the male are fewer in number, being fourteen pairs of preanal papillae, against thirty-two in *A. varani*, and three pairs of postanal against five in *A. varani*.

It is interesting to note the presence in some individuals of two intestinal caeca, and it appears that this is a character in which some individual variation may be expected to occur. Baylis (1921) has drawn attention to a somewhat similar variation in *Polydelphis quadricornis* and (1922) in *P. sewelli*, in which two species only some individuals showed the presence of this intestinal appendage.

STRONGYLURIS BREVICAUDATA, Müller, 1894

Host :—*Agama colonorum*. Position :—Intestine. Locality :—Northern Nigeria.

Body tapers gradually from the middle to the head, and measures 5·5 to 12 mm. in length. The cuticle is thick and marked with fine transverse striations and carries numerous minute papillae arranged at fairly regular intervals in longitudinal lines ; the intervals between the papillae increase towards the posterior extremity. These papillae are much more clearly seen in some specimens than in others, but I have not seen any special crown of papillae below the head. The head (fig. 9) is formed by three lips and is narrower than the neck immediately behind, so that a ' shoulder ' is formed. Each of the three lips carries a pair of papillae, while at the anterior edge is a very delicate membrane which can only be seen when the lips are extended forward ; in specimens where the lips are closed together this membrane is out of sight, but seen on edge when extended, it gives to the lip a hooked appearance. Behind the mouth is a well-developed pharynx (fig. 10) 0·18 to 0·23 mm. in length ; at its junction with the second part of the oesophagus is a small cavity in the form of a kink in the lumen of the tube. The second portion of the oesophagus usually describes one or two wide curves before reaching the bulb at the posterior end ; the length of the entire

oesophagus is 1·8 to 2·4 mm., from 1/5·2 to 1/3 of the length of the whole worm. The nerve ring is placed about twice the length of the pharynx from the head. The intestine, at its junction with the oesophagus, is considerably wider than the oesophageal bulb.

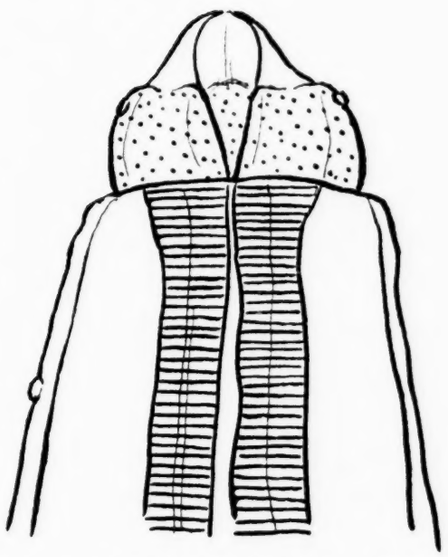

FIG. 9. *Strongyluris brevicaudata.* Anterior extremity. × 333.

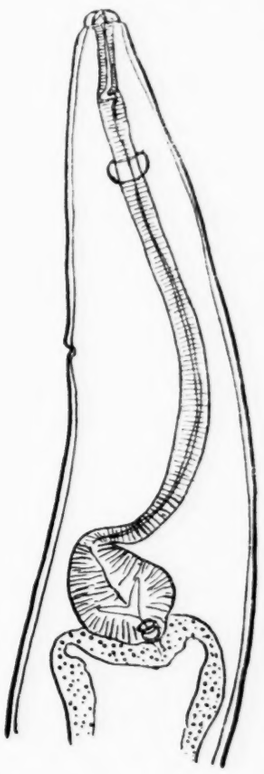

FIG. 10. *Strongyluris brevicaudata.* Oesophageal portion. × 40.

The male measures 5·5 to 9·6 mm. in length and 0·33 to 0·6 mm. in its widest part. The caudal alae (fig. 11) are wide and short, and

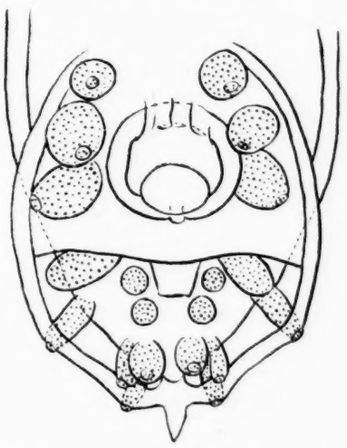

FIG. 11. *Strongyluris brevicaudata.* Caudal extremity of male, ventral view. × 125.

have thickened margins which almost meet on the ventral surface in front of the preanal sucker. The papillae number ten pairs. Of these, three are placed at the side of the preanal sucker, the anterior one is often small and sometimes absent, while the middle and posterior ones are large flask-shaped papillae. Of the remaining seven pairs, two sessile pairs are placed ventrally at the side of the anus, while two pedunculated pairs are placed lateral to these, the other three pairs of papillae being grouped on either side of the extremity. Some specimens show one or two very small, sessile papillae subventrally placed in front of the anus. The length of the caudal extremity behind the cloaca is 0·07 to 0·11 mm. (1/105 to 1/75 of the length of the whole worm) ; it has a small spike at the extreme end. The preanal sucker has a strongly chitinised rim and is circular in outline, measuring 0·12 mm. in diameter. The spicules are in the form of long, tapering tubes of equal measurement from 1·5 to 1·95 mm. (1/5·6 to 1/3·6 of the body length) and have a reticulated surface.

The female measures 6·75 to 12 mm. in length and 0·345 to 0·855 mm. across the widest part ; the vulva is situated posterior to the middle and takes the form of a transverse slit with salient lips. The vagina describes a very winding course ; after first taking a forward direction for about 0·42 mm., it bends backwards, dividing into the two branches of the uterus ; these continue to a point about 1·2 mm. from the caudal extremity, where they bend forward again to reach the ovaries, which are situated in the anterior part of the body. The caudal extremity of the female is in the form of a short cone 0·12 to 0·21 mm. long from anus to extremity and carries two small papillae. The eggs are oval, about 73μ by 43μ in size, unsegmented, and with a thick, smooth shell.

Details of measurement of closely allied members of the genus *Strongyluris* are compiled in Table I. The morphological characters of these seven species, as described by the various observers, are practically identical ; *S. gigas* is the only species showing marked variation in detailed measurements from the one described above, while *S. calotis*, *S. ornata* (Gendre) and *S. ornata* (Linst.) only show variation in the ratio of spicule length to body length. It seems more than likely that the strongylurids described under the specific names *chamaelonis*, *streptoesophagus* and *brevicaudata* are one and the

Name	*S. brevicaudata*, Müller [Spaul, 1923]		*S. brevicaudata*, Müller here described		*S. streptocephagus*, Connall, 1912		*S. chamaeleontis* Baylis & Daubney, 1922		*S. calotis* Baylis & Daubney, 1923		*S. ornata* (Gendre) [Spaul, 1923]		*S. ornata* (Linst.) [Spaul, 1923]		*S. gigas*, Spaul, 1923	
Host	*Agama*, sp.		*Agama colonorum*		*Agama colonorum*		*Chamaelon vulgaris*		*Calotis nigrilabris*		*Agama colonorum*		*Stello vulgaris*		*Agama distanti*	
Locality	East Africa		West Africa		West Africa		Calcutta		Ceylon		Africa		Madagascar		East Africa.	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Length	9.6	10.5	5.5 – 9.6	6.75 – 12	8.6	9.1	6.3	8.4 – 8.75	8.9 – 11.1	11 – 13.65	12 – 15	13 – 17	12	...	32	35
Breadth	0.45	0.55	0.33 – 0.6	0.345 – 0.855	0.44	0.5	0.5 – 0.7		0.4 – 0.5	0.55–0.75	0.76–0.84	0.9 – 1	0.83	...	1.2 – 1.5	1.5
Length of tail	0.1	0.2	0.07 – 0.11	0.12 – 0.21	0.01	0.2	0.13	0.3	0.1–0.12	0.2 – 0.25	...	...	...	...	0.25	0.4
Relative length of tail	$\frac{1}{95}$	$\frac{1}{50}$	$\frac{1}{105} - \frac{1}{71}$	$\frac{1}{85} - \frac{1}{40}$	$\frac{1}{860}$	$\frac{1}{45}$	$\frac{1}{48}$	$\frac{1}{29}$	$\frac{1}{90} - \frac{1}{89}$	$\frac{1}{55} - \frac{1}{54}$	$\frac{1}{93} - \frac{1}{90}$	$\frac{1}{67} - \frac{1}{61}$	$\frac{1}{100}$	$\frac{1}{60}$	$\frac{1}{125}$	$\frac{1}{90}$
Length of pharynx	0.25	0.29	0.18 – 0.22	0.20 – 0.23	0.23	...	0.18 – 0.22		0.26 – 0.3	...	...	...	...	...	0.3	0.4
Length of oesophagus	1.9	2	1.8 – 2.17	1.8 – 2.4	1.58	1.95	1.1	1.45	1.75 – 2.25		...	...	...	...	3	3.5
Relative length of oesophagus	$\frac{1}{5.1}$	$\frac{1}{5.25}$	$\frac{1}{4.9} - \frac{1}{3}$	$\frac{1}{5.2} - \frac{1}{3.6}$	$\frac{1}{5.4}$	$\frac{1}{4.6}$	$\frac{1}{5.7}$	$\frac{1}{6.0}$	$\frac{1}{6.3}$	$\frac{1}{6}$	$\frac{1}{5.6} - \frac{1}{4.7}$	$\frac{1}{5.3} - \frac{1}{5}$	$\frac{1}{6}$	...	$\frac{1}{12} - \frac{1}{10}$	$\frac{1}{10} - \frac{1}{9}$
Length of spicules	1.3	...	1.5 – 1.95	...	1.2	...	1.1	...	0.75 – 0.8	...	1.5	...	1.06	...	1.1	...
Relative length of spicules	$\frac{1}{7.4}$	...	$\frac{1}{5.6} - \frac{1}{3.6}$	...	7	...	$\frac{1}{5.7}$	...	$\frac{1}{14}$	...	$\frac{1}{10}$	...	$\frac{1}{11}$	...	$\frac{1}{29}$	...
Width of oesophagus	0.04	0.07	0.043 – 0.068	0.06 – 0.085	0.08	0.07	...	...	...	...	...	...	...	...	0.085	0.096
Vulva from post. ext.	...	3.5	...	3.75 – 6.75	...	3.35	...	3 – 3.3	...	4.7 – 5.65	...	...	...	...	...	17
Ratio of above measurement to body length	...	$\frac{10}{30}$	...	$\frac{10}{22} - \frac{10}{27}$	...	$\frac{10}{27}$	...	$\frac{10}{26}$	...	$\frac{10}{24}$	...	23	...	$\frac{10}{20}$	...	$\frac{10}{21}$
Sucker from post. ext.	...	...	0.2	...	0.76	...	...	...	...	...	...	...	...	...	0.4	...
Diameter of head	...	...	0.07 – 0.08		...	...	...	...	0.06 – 0.08		...	...	...	...	...	...
Eggs, length	...	63μ	...	73μ	...	60μ	...	87.5μ	...	87.5μ – 97.5μ	...	76μ	...	70μ	...	150μ
Eggs, breadth	...	48μ	...	43μ	...	30μ	...	55μ	...	50μ	...	44μ	...	47μ	...	90μ

All the above figures are in mm. unless otherwise stated.

same species, while it is not improbable that the species *calotis*, *ornata* (Gendre) and *ornata* (Linst.) will also prove to be identical with the genotype *S. brevicaudata*.

AFRICANA AFRICANA (Gendre)

Host :—Lizard (species of skink) and *Bufo regularis*. Locality :—Northern Nigeria.

This worm is of small size, 3·17 to 5·77 mm. long ; the cuticle, which is thin, is marked with fine cross striations ; longitudinal lines in the musculature show clearly through ; there are two narrow lateral flanges 19 to 29μ in width which terminate a short distance from either extremity ; a few minute papillae may be seen on the body surface. The mouth is surrounded by three subglobular lips (fig. 12), each bearing a pair of papillae, and separated

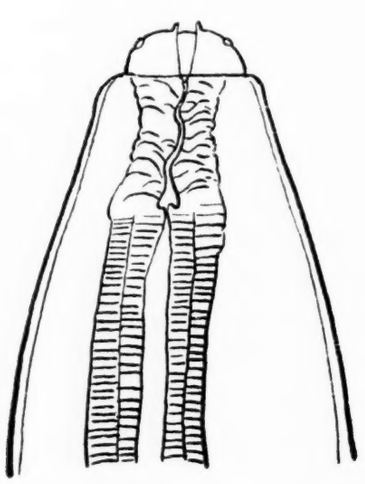

FIG. 12. *Africana africana*. Anterior extremity. × 253.

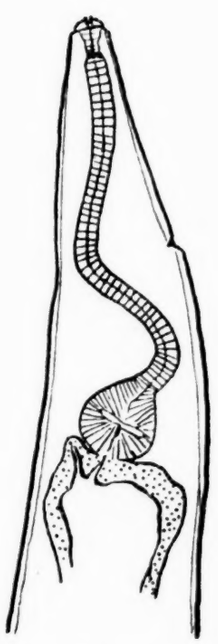

FIG. 13. *Africana africana*. Oesophageal portion. × 53.

from the neck by a well-marked line ; the diameter of the head is 50 to 68μ and its depth 26 to 29μ. The excretory pore is situated about half-way down the oesophageal portion of the body. Following the small infundibuliform mouth cavity is a short pharynx 46 to 60μ in length, which widens posteriorly to form a small cavity, but the

ventral kink seen so plainly in *Strongyluris* is not present. The oesophagus (fig. 13) 1·05 to 1·2 mm. in its entire length, usually describes two curves between the pharynx and the bulb ; this latter structure is well-developed and measures about 0·200 by 0·180 mm. ; the intestine at its junction with the oesophagus is wider than the bulb.

Male, 4·5 to 4·95 mm. in length and 0·321 to 0·37 mm. at its widest measurement ; it has two slightly unequal spicules varying in length from 1·55 to 1·73 mm. and up to 0·846 mm. in breadth at their widest part ; there is a ventral notch at the proximal end, and each spicule presents two ventral gutters running parallel throughout the greater part of its length to a point near the apex ; the surface of the spicules is reticulated. Caudal alae are present, apart from the lateral flanges, and measure about 0·075 mm. at their widest part, which is about the level of the preanal sucker. The tail (figs. 14 and 15) is

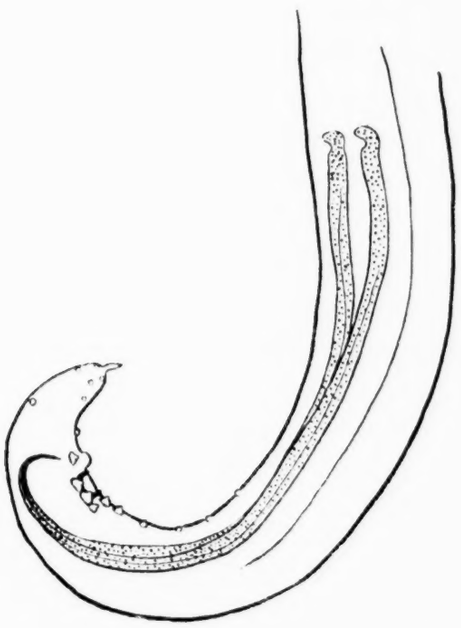

FIG. 14. *Africana africana.* Caudal extremity of male, lateral view. × 55.

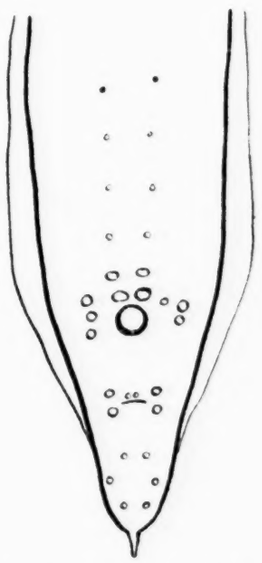

FIG. 15. *Africana africana.* Caudal extremity of male, ventral view. × 80.

conical and ventrally curved ; it measures 0·21 to 0·28 mm. from the cloaca to the extremity which carries a small spike. The caudal papillae number nineteen pairs, twelve preanal, four postanal and three dorsal ; of the preanal there is a subventral line of six in front

of the preanal sucker ; these increase in size from before back, three other pairs are placed subventrally at the side of the sucker, one pair almost laterally, mid-way between sucker and cloaca,* one pair subventrally immediately in front of the cloaca, and one very small pair ventrally on the prominence just in front of the cloaca. Of the postanal papillae three pairs form a ring on the ventral and subventral surface, while the fourth pair is placed subventrally just behind the anus. The three dorsal papillae are posterior to the anus. The preanal sucker is circular in outline, 55 to 66μ in diameter and has a well-fortified chitinous rim.

The female measures 3·15 mm. to 5·77 mm. in length and 0·21 to 0·375 mm. in maximum diameter ; the vulva, which is not salient, is situated anterior to the middle of the body, 1·8 to 3·67 mm. from the posterior extremity ; the vagina follows a winding course, but in general runs back from the vulva ; the ovaries are situated in the anterior part of the body. The caudal extremity is 0·22 to 0·34 mm. in length and tapers off to a very fine point. The eggs have a thick smooth shell and measure 66 by 42μ to 86 by 46μ ; they are not segmented when laid.

Table No. II gives detailed measurements from the three known species of this genus, *A. africana*, Gendre (1909), *A. acuticeps* and *A. brodeni*, Gedoelst (1916) with measurements from the worm above described. These species seem to be very closely allied and the only difference of any diagnostic value seems to be the number and arrangement of papillae on the caudal extremity of the male.

* By an oversight I have omitted this papilla in making the final drawing for fig. 15.

TABLE II.

Details of measurements of the species of *Africana*.

Name	*A. africana* (Gendre, 1909)		*A. africana* (Gendre, 1909) measurements made from worm described in text		*A. acuticeps* (Gedoelst, 1916)		*A. brodeni* (Gedoelst, 1916)	
Length	♂ 5 – 7	♀ 6.7 – 8	♂ 4.5 – 4.95	♀ 3.15–5.77	♂ 6.5 – 7	♀ 6.8 – 8.5	♂ 6.5	♀ 7.8
Breadth	0.36–0.46	0.42–0.48	0.321–0.37	0.21–0.375	0.385–0.39	0.4–0.415	0.45–0.465	0.385
Depth of head ...	...	...	23–29μ	26–29μ	...	20μ	...	20μ
Diameter of head ...	...	...	50μ	50–68μ	...	47–52μ	...	56μ
Length of pharynx ...	...	...	46–50μ	48–60μ	65–70μ	...	72μ	...
Length of oesophagus	...	...	1.12–1.14	1.05–1.20	...	1.00	...	1.28
Ratio to entire worm	$\frac{1}{3.8}$ – $\frac{1}{4}$	$\frac{1}{4}$ – $\frac{1}{4.4}$	$\frac{1}{4}$ – $\frac{1}{4.3}$	$\frac{1}{3}$ – $\frac{1}{4.8}$	$\frac{1}{5}$	$\frac{1}{5.5}$	...	$\frac{1}{4.1}$
Length of bulb ...	...	...	...	200μ	...	200–230μ	...	...
Width of bulb ...	...	...	...	180μ	...	170–180μ	...	185μ
Length of tail ...	...	...	0.21–0.28	0.22–0.34	0.24 0.25	0.30–0.34	0.29	0.36
Ratio to entire worm	$\frac{1}{19.5}$ – $\frac{1}{23}$	$\frac{1}{17}$ – $\frac{1}{17.5}$	$\frac{1}{21}$ – $\frac{1}{18}$	$\frac{1}{14.5}$ – $\frac{1}{17}$	$\frac{1}{21}$ – $\frac{1}{28}$	$\frac{1}{22}$ – $\frac{1}{25}$	$\frac{1}{21}$	$\frac{1}{21}$
Length of spicules ...	1+	...	1.55–1.73	...	1.8 and 2.0	...	1.85 and 1.69	...
Ratio to entire worm	$\frac{1}{5}$	...	$\frac{1}{2.9}$ – $\frac{1}{2.8}$	...	$\frac{1}{3.5}$	...	$\frac{1}{3.5}$	...
Diameter of sucker ...	...	...	63–66μ	...	64μ	...	55μ	...
Distance of vulva from posterior end	...	...	...	1.8–3.67	...	...	...	...
Ratio of above to body length	...	$\frac{10}{16.6}$	...	$\frac{10}{17}$ – $\frac{10}{16}$	...	$\frac{10}{17}$	...	$\frac{10}{14}$
Eggs, length	...	60–66μ	...	66–86μ	...	64–68μ	...	75–80μ
Eggs, breadth ...	...	39–41μ	...	44–46μ	...	40–44μ	...	48–52μ

All the above figures are in mm. unless otherwise stated.

OXYURIS PRAEPUTIALIS Skrjabin, 1914

Host :—*Bufo regularis*. Locality :—Nigeria.

Unfortunately only female specimens were available, and as the male has not yet been described, the species cannot be referred to its proper genus. The characters presented by the female are as follows :—

Body white, 2·85 to 4·95 mm. in length by 0·21 to 0·435 mm. in width at its broadest part ; it tapers from the level of the oesophageal bulb towards the head and is usually coiled, or twisted into the form of the letter ' S.' The cuticle is crossed with very fine striations and bears a few minute papillae in the cervical region. The posterior extremity (fig. 16) tapers rapidly behind the anus, and terminates in

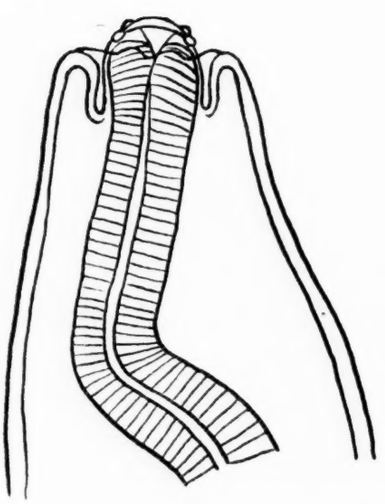

FIG. 16. *Oxyuris praeputialis*. Anterior extremity, head retracted. × 253.

FIG. 17. *Oxyuris praeputialis*. Posterior extremity of female. × 73.

a very fine, straight spike, from 0·24 to 0·495 mm. in length. Small lateral flanges are present, commencing about 0·06 mm. behind the head and terminating about the same distance from the spike at the caudal extremity. The head (fig. 17) is formed by three lips 0·06 to 0·083 mm. wide, each bearing two comparatively large papillae and a short, anterior, marginal membrane. The whole head seems to

be capable of withdrawal within a cuticular fold of the neck and in some specimens it is almost completely hidden there. The nerve ring is situated about 0·16 mm. from the anterior extremity and the excretory pore 0·3 to 0·36 mm. from the same point.

In specimens where the head is retracted, the first portion of the oesophagus is always bent in the form of a double curve ; its diameter is 0·053 to 0·06 mm. and the muscular portion seems to run well up into the bases of the lips. The oesophageal bulb is a well-formed structure, more or less spherical in shape and measures about 0·13 mm. in diameter ; the complete length of the oesophagus is 0·42 to 0·60 mm. The vulva is placed anterior to the middle of the body, 1·85 to 2·7 mm. from the posterior extremity, tracing the vagina inwards it is found, after a short forward bend to turn backward, in which direction it continues to a point near the anus ; the ovaries are situated in the anterior part of the body.

The worm is ovoviviparous ; many specimens were found to contain numerous larvae.

TRICURIS DISCOLOR (Linstow, 1909)

Host :—Cow. Position :—Intestine. Locality :—Lancashire.

Body white, cuticle ringed with striations at intervals of about 7·5μ, contour of the narrow anterior part serrate, two lateral vesicular swellings may be seen at the head.

The male measures 50·25 to 58·75 mm. in length ; the narrow oesophageal portion is 33·75 to 38·5 mm. long and 0·175 mm. broad, and represents about two-thirds of the complete length of the worm ; the thicker posterior portion is 16·5 to 20·25 mm. long and 0·55 to 0·75 mm. at its widest part. The spicule measures 1·95 to 2·3 mm. in length and only 10·0 to 11·5μ in thickness, being less than half the size of that of *T. ovis* in both measurements (fig. 18). The spicule sheath has a diameter of 165μ and is covered with spines, these being larger in size and not so closely spaced as in *T. ovis*. Although no specimens were found with spicule extruded, nothing was visible to correspond with the expanded portion at the extremity of the evaginated spicule sheath of *T. ovis*.

The female measures 43·00 to 51·75 mm. in length, the anterior portion is 33·00 to 39·00 mm. long and 0·165 mm. broad and

represents three-quarters of the entire length ; the thicker posterior portion is 10·00 to 12·75 mm. long and 0·825 mm. broad. The egg measures 60 by 30μ to 73 by 33μ, and, excluding the opercula, 43 to 46μ.

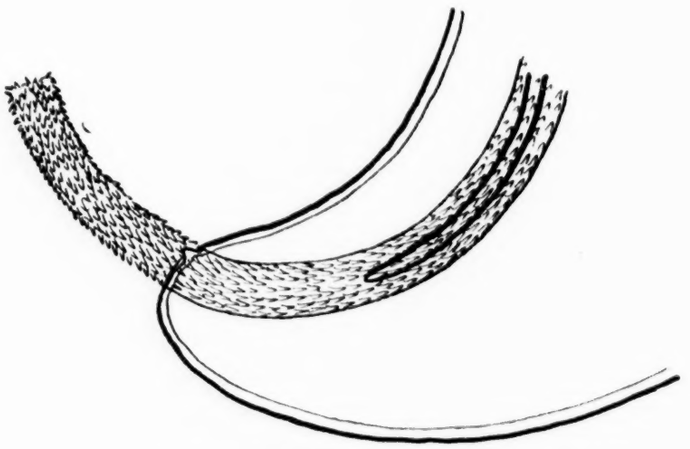

FIG. 18. *Tricuris discolor*. Caudal extremity of male, lateral view. × 190.

So far as can be ascertained, this species has not previously been recorded in this country.

PHYSALOPTERA PRAEPUTIALIS v. Linstow, 1889

Synonym :—*Chlamydonema felineus* Hegt, 1910

Material : four female specimens from the dog corresponding in every way with the description of this species.

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ON THE HIBERNATION OF ADULT MOSQUITOS

BY

W. REES WRIGHT, B.Sc.

(*Received for publication* 10 *November*, 1924)

The investigations of which the results form the subject of this paper were carried out during the years 1920-24 in a district of North Carnarvonshire lying between the Menai Straits and the Snowdonian mountains. The purpose of the investigations was to endeavour to elucidate the habits of hibernating mosquitos and the relation of certain species of mosquito to man and to his domestic stock.

The field work involved consisted of visiting farm buildings of all types and collecting, for identification and also for use in other studies, the mosquitos seen. The insects were collected in 6-in. by 1-in. specimen tubes; the tube was brought over a resting mosquito, which on taking flight generally flew up into the tube, when it could be secured by means of a wad of cotton wool. Owing to the poor lighting of most of the buildings, the work had to be done in most cases by the light of an electric torch. For a short time a glass tube with gauze covering one end, the other being closed with a rubber 'valve' (as devised by Adie, 1911) was used; it was found that under the cool conditions in which the collecting often was carried out, moisture from the breath condensed on the inner wall of the tube in quantity sufficient to imperil the safety of the insects. It should be remarked that this type of tube has proved of great use in the laboratory as a 'cage,' since the 'valve' enables one to release mosquitos into the tube, or to remove them from it, much more easily than is the case with a tube closed with gauze at both ends.

In the investigation of such a matter as the hibernation of animals, there are two sets of factors which must especially be considered, viz., climatological factors, and environmental factors. Taking the climatological factors first, it may be said that North Carnarvonshire has a climate which is mild but somewhat wet.

The difference between the isotherm for the months of January and July is about 10° C., the former being approximately 5·5° C., and the latter a trifle over 15° C. Away from the Snowdonian mountains a severe frost or a fall of snow is uncommon, while light frosts—sufficient to freeze up road puddles—are not often experienced. It is probable that the relatively slight range of temperature explains certain differences between the results set forth here and those obtained by Sella (1920) and Grassi (1921a and 1923) among others. A temperature of 5° C. is probably less rigorous for a race of animals accustomed to a temperature of only 15° C. than for a race accustomed to something in the neighbourhood of 25° C.

The second group of factors largely resolves itself, in the present instance, into the conditions of the farm buildings and of the animals found therein. The main agricultural pursuit of the area is the grazing of sheep ; while dairy farming is carried out on a small scale, there are only two or three large dairy-farms in the district examined. On a typical farm we find up to a dozen cows, lodged probably in one shed, a few calves in another shed (a ' calf-cot '), two or three horses in their stable, and usually a dozen or more pigs in three or four styes. While practically all farmers keep fowls, it is only a few that do so on any considerable scale. The farm buildings are for the most part old, apart from the dwelling-houses, which in most cases are of far later date—the old dwelling-houses were usually part of the same range of buildings as the animal houses. In consequence of their age, the animal houses are dark, low, ill-ventilated and warm, and often difficult to keep clean. This is more especially the case with the pig-styes and calf-cots and to a lesser degree with the stables. While the other buildings have a flat ceiling—often forming the floor of a loft above—the pig-styes are almost all lean-to in construction ; about thirty inches high in front and six to eight feet high at the back ; very rarely the doorway is placed in the high side, or in the end, but it is even then no larger than will admit a large pig. There is, in consequence, a considerable space overhead in which warm air can accumulate. Except the pig-styes, which are hardly touched, the buildings are whitewashed once a year on most farms, when they get a thorough cleaning ; this usually takes place in early summer. Throughout the winter the stables and cowsheds are cleaned daily ; the other buildings are attended to less frequently or not at all.

The pig-styes generally have masses of cobwebs dependent from their ceilings.

Throughout Northern Europe only three species of mosquito are known to hibernate as the adult, viz., *Anopheles maculipennis*, *Theobaldia annulata* and *Culex pipiens*. In the autumn of 1920, when I commenced my investigations, our knowledge of the phenomena of hibernation was not far advanced from the point where Annett and Dutton left it in 1901. Thus Hindle (1914) briefly states that

> 'In cold regions mosquitos pass through the winter in the egg-stage, but in addition some of the females hibernate in dark corners.'

Patton and Cragg (1913) state that

> 'Mosquitos, like all other insects which normally have a short life-history and which can only multiply under certain conditions are able to exist . . . . during unfavourable seasons. . . . . The commonest manner in which this is brought about is by the hibernation of impregnated females, which, finding the season too advanced to complete the maturation and deposition of their ova, seek out resting places and remain concealed until favourable conditions present themselves at the commencement of the next season. During this period, which may extend to several months in temperate climates such as those of Europe, they feed seldom if at all, and remain in a passive and torpid condition, living upon the store of food material already accumulated, until they are revived by the warm weather.'

Finally, Lang (1918) only cites Annett and Dutton's work on hibernation.

Since the autumn of 1920, a number of excellent papers on the hibernation of mosquitos have appeared; to mention but a few, those of Grassi and of Sella from Italy (*op. cit. supra*), of Osterwald and Tänzer (1920) from Saxony, and probably most important of all, Wesenburg-Lund's memoir on the Danish mosquitos. There have been close approximations between the results obtained by the more Northern workers—*e.g.* Wesenburg-Lund (1921), Osterwald and Tänzer, and myself, and between the results of more Southern workers such as Sella and Grassi; as I have suggested above, a few variations, which will be pointed out in due course, are probably climatic in origin.

The three species of mosquito which have already been mentioned are found in North Carnarvonshire. In the summer the larvae are to be found in almost every ditch, *Anopheles* being particularly abundant. During the winter neither of the Culicine species are as abundant as one would expect to find them. In consequence of this,

and also because of its far greater importance from the point of view of medicine, most attention has been paid to *Anopheles*.

Roubaud has recently (1923) proposed a physiological basis for hibernation. He suggests that the hibernation is brought about, not by the increasing cold, but by an auto-intoxication of the insects by a ' renal surcharge.' He believes that the latest-hatched members of the summer generations inherit a ' patrimoine d'intoxication ' which gives rise to the peculiar physiological conditions of the hibernating female. Among others, this condition manifests itself physically by a hypertrophy of the malpighian tubules, physiologically by the fact that any blood ingested goes to form fat body, instead of assisting in the maturation of the ova. It is only when this intoxication has worked itself off, which does not happen till early spring, that the eggs develop. As far as North Wales is concerned, I have not observed either the physical or physiological signs of this intoxication ; out of numbers of mosquitos dissected during the winter months, in none did I observe any abnormal state of the malpighian tubules, while I discovered that both in the laboratory and also under natural conditions, female mosquitos matured their eggs as early as January.

Grassi (1921a) and also Sella (1920) have drawn a distinction between true or rigorous hibernation and partial hibernation. The former is defined as a condition in which the insect develops fat-body towards the late autumn, and does not then feed until spring, while in the latter stage it continues to feed and does not become fat. Grassi believes that it is the former class of females that perpetuates the species, and that the latter die off before the spring. According to Roubaud (1923) this is also the case in France. In the former state the insect does not move about. Whatever may be the conditions on the European continent, where the mosquitos are subjected to a greater variation of temperature than occurs in this country, in Carnarvonshire I found *Anopheles* flying actively within the farm buildings throughout the winter, save when the temperature fell to somewhere near zero Centigrade. The insect is willing to feed at all times except immediately after a blood meal. Yorke and Macfie, experimenting at the Liverpool School of Tropical Medicine with *Anopheles* which I had sent them from North Wales, found that a considerable number died in their incubators, apparently as the

result of the development of their eggs, which they could not deposit owing to lack of facilities. I have observed the development of eggs in wild *Theobaldia* as early as January.

Anopheles maculipennis. This species is, throughout the year, an inhabitant of farm buildings, as Roubaud (1920) and Wesenburg-Lund (1921), among others, have pointed out. During the winter females alone occur.

It occurs chiefly in the pig-styes ; next, in order of frequency, in calf-cots, cowsheds and stables—very rarely in these last. In lofts and barns it rarely occurs—never, I think, unless these are in communication with a chamber containing animals. I have only once found it in a fowl-house. Taking at random from my notes a series of twelve farms examined during the latter part of November and the first part of December, 1923, I find that I recorded it as occurring abundantly in six groups of pig-styes, and as being less abundant in four other groups. It was once recorded as abundant in cowsheds, and four times as occurring in these buildings. It was once recorded from a stable, and twice from lofts or barns. At one farm where it was absent from the pig-styes, these had been empty for at least two months, while at one farm there were no buildings other than cowsheds and their lofts. It occurred in pig-styes about five times ; if a longer series of records were to be taken, the attraction which pig-styes have for mosquitos would be even more marked.

In whatever buildings *Anopheles maculipennis* occurs, it is more abundant towards the upper parts, as was pointed out by Annett and Dutton (1901). Frequently it rests on cobwebs. As a general rule, it will be found in the more dimly-lit places, such as the sides of beams furthest from the light. As has already been stated, it feeds throughout the winter ; and at least 50 per cent. of the mosquitos collected during these investigations contained recognisable blood.

Usually the insects take up the resting-position so often described, but, as Annett and Dutton pointed out, under certain conditions, especially cold, they take up a *Culex*-like attitude. Under the influence of cold they become exceedingly torpid and when touched make no effort to fly. They rapidly recover in a warmer place, however. Normally the insects are active, flying spontaneously

both in captivity and in nature. A bright light, as well as the approach of some object, causes them to take flight; in this my experience has been different to that of Roubaud (1918) who found that a bright light caused flying mosquitos to come to rest.

The attraction which the animals' quarters have for this mosquito is probably due to several causes. I am of opinion that the insect's main desiderata are darkness, warmth, and easily-accessible food. This last condition will be fulfilled by dwelling-houses as well as animal houses, and need not be taken into consideration. In the area with which I am acquainted, it is the pig-styes which fulfil the first two conditions most completely, while the animal houses as a whole are darker, warmer and quieter than the dwelling-houses. It may be that an attraction towards the animal houses arising from these causes may be in progress of giving rise to a zoophile race of mosquitos, as has been supposed by Roubaud (1920) and by Grassi (1921b). It is possible that the comparative hairlessness of the pig, and the fact that its skin, though thick, is highly vascular—affording a readily-tapped source of blood—may be additional attractions for the insect.

The Malaria Committee for the Province of North Holland (1920) found that mosquitos were most numerous in pig-styes. On the other hand Vogel (1921), working in the zone occupied by the Fifth German Army during the late War, found that cowsheds were preferred to pig-styes.

Theobaldia annulata. This species also hibernates in the adult stage, the females alone surviving the winter. Like *Anopheles* it is an inhabitant of the animal quarters, but occurs also in the colder buildings, such as store-houses, etc., and occasionally in dwelling-houses. I think, however, that it resorts to these last only when no other buildings where food may be obtained are available. Like *Anopheles*, it feeds throughout the winter. It rests on cobwebs as a rule, on the lower part of a sloping ceiling or on the wall, and takes up the hunchback *Culex* attitude.

On 10th January, 1924, I obtained, in some cellars at the University College of North Wales, Bangor, two females of this species which contained matured eggs. They were given a blood meal, and placed in a breeding cage, where both oviposited the same

day ; the temperature of the laboratory was about 13° Centigrade. The larvae hatched out on the 21st, the maximum temperature in the interim being 14·7° C. and the minimum 6·7° C.—mean temperature about 13° C. Unfortunately, the larvae were destroyed through an accident to the breeding tank.

In 1924, both this species and *Anopheles* left the farm buildings towards the end of March, but larvae were not found till mid-May.

Culex pipiens. Unlike the two previously-mentioned species, *Culex* hibernates almost exclusively in cellars, lofts and similar cool places. It is a more lethargic insect than the other two, spending most of its time resting on the walls and on cobwebs ; it is found towards the lower part of the walls. When disturbed it flies actively. This species is supposed to be more partial to avian blood than to mammalian. I have only rarely taken specimens containing blood, and, unfortunately, on those occasions I have had no facilities for determining the nature of the blood, whether mammalian or avian. In the laboratory this species will not feed on man in my experience.

During 1923-24 my attention was directed, in addition to the main investigation, towards the possibility of reducing the number of *Anopheles* in a district, by the collection or destruction of the hibernating adults. One farm was particularly well-adapted for investigations of this nature, since the breeding-place of the mosquitos found in it was known to be a small pond actually within the farmyard, and there were no other places near-by—either hibernating quarters or breeding-places—from which an influx of mosquitos might be expected. All mosquitos seen were collected—many hundred *Anopheles* and a few *Culex* and *Theobaldia*. In the course of this work it was found that if a building, *e.g.*, a pig-stye, was apparently cleared of mosquitos one day, more would be found there the next. Having regard to the construction of the buildings, I am inclined to interpret this as being due to emergence of mosquitos from recesses in which they could not be detected, rather than to an actual migration ; a certain amount of migration between adjoining buildings undoubtedly takes place, when there are any direct communications such as holes in the walls. Buildings were apparently cleared by several collections.

Despite the collecting, *Anopheles*, *Theobaldia* and *Culex* appeared

in these breeding-places in early summer, and were abundant in the buildings, and I am forced to the conclusion that the collection of hibernating mosquitos is by itself of no value as a means of control

SUMMARY

1. *Anopheles maculipennis*, *Theobaldia annulata* and *Culex pipiens* hibernate as adult females.

2. *Anopheles maculipennis* occurs in those farm buildings which contain animals, especially in pig-styes ; it occurs also in calf-cots, cowsheds and stables, and, very occasionally, in lofts or barns. When these communicate with a building containing animals, the anopheline flies spontaneously at any time of day. It feeds throughout the winter. Under laboratory conditions, the females will develop eggs during the winter, if kept at a sufficiently high temperature.

3. *Theobaldia annulata* occurs in the same type of building as does *Anopheles* and also in cooler places such as lofts and cellars. Like *Anopheles*, it feeds throughout the winter and flies spontaneously. Females which contain matured eggs have been caught in January.

4. *Culex pipiens* occurs in cool buildings and cellars exclusively. It is much less active than the other two species.

5. Experiments to determine the effect of collecting the hibernating females have shown that this alone is not a useful means of control.

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I have very great pleasure in taking this opportunity of acknowledging my indebtedness to the agricultural population of the district, employers and employed alike, for affording me all possible facilities and assistance for the prosecution of my researches ; to my late chiefs at the University College of North Wales, Bangor (where these investigations were carred out), Professor P. J. White, M.B., F.R.S.E., Department of Zoology, and C. L. Walton, Esq., M.Sc., Ph.D., Adviser in Agricultural Zoology, Department of Agriculture, for assistance and advice ; to Professor Warrington Yorke, M.D.,

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MISCELLANEA

CORRIGENDA

" Descriptions of new mosquitos from South America "
(*Ann. Trop. Med. & Parasit.*, Vol. XVIII, No. 3, pp. 363-375).

Culex (*Choeroporpa*) *innominatus*, sp.n. Evans (*loc. cit.*, p. 365).
For ' paratypes ' and ' cotypes ' respectively, read :
' Cotypes : 2♀♀ River Amazon, 1915 (Dr. Aiken Clark) ; 2♂♂ Palo Negro, Venezuela, 30.8.22, and 1♂ Mariara, Estada Carabobo, Venezuela, 11.8.22 (Dr. M. Núñez Tovar).'

Culex (*Choeroporpa*) *clarki*, sp.n. Evans (*loc. cit.*, p. 367).
For ' paratypes ' read ' cotypes.'

Culex innovator, sp.n. Evans (*loc. cit.*, p. 375).
For ' paratypes ' read ' cotypes.'

ALWEN M. EVANS,
10*th November*, 1924.

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> Robinson, S. (1914). 'The spleen in malaria.' *Annals of Nosology*, Vol. XX, pp. 20-25.
>
> Smith, J. (1900). 'Enlargement of the spleen in malaria.' *Journal of Pathometry*, Vol. I, pp. 1-20.

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SOME CESTODA DESCRIBED BY BEDDARD, 1911-1920

BY

JEAN G. BAER, D.Sc.

(*Zoological Institute, University of Neuchâtel, Switzerland*)

(*Received for publication* 5 *December*, 1924)

Between the years 1911 and 1920, Professor Beddard published in the *Proceedings of the Zoological Society of London* a series of papers dealing with new and known species of Cestoda collected from the animals dying in the Society's Gardens.

A few years ago, Professor Fuhrmann obtained the loan of some of Professor Beddard's types and co-types with the intention of re-examining and eventually redescribing them. However, more important matters having delayed this work, Professor Fuhrmann asked me to undertake it. I tender my sincerest thanks to Professor Fuhrmann for this material, as well as for his kindly advice.

The Cestoda of which we have been able to examine the types or co-types are the following. The names printed in small italics must fall and be considered as synonyms.

1. *ANOPLOTAENIA DASYURI*, Beddard, 1911.
2. *DASYUROTAENIA ROBUSTUS*, Beddard, 1912.
3. *Hyracotaenia hyracis*, Beddard, 1912.
4. *Hyracotaenia procaviae*, Beddard, 1912.
5. *Inermicapsifer capensis*, Beddard, 1912.
6. *Monoecocestus erethizontis*, Beddard, 1914.
7. *Otidiotaenia eupoditis*, Beddard, 1912.
8. *Thysanotaenia gambianum* (Beddard, 1911).
9. *THYSANOTAENIA LEMURIS*, Beddard, 1911.

We have ourselves already re-examined Nos. 3, 4, 5, and 8, and have published our results in a preliminary report (1924). Our conclusions were as follows: *Hyracotaenia hyracis* = *Inermicapsifer capensis* = *Inermicapsifer hyracis* (Rudolphi, 1810), *Hyracotaenia procaviae* = *Inermicapsifer pagenstecheri* (Setti, 1897), and *Thysanotaenia gambianum* = *Inermicapsifer guineensis* (Graham, 1908). No. 7 has

been re-examined by Skrjabin (1914) who finds *Otidiotaenia eupoditis* to be a synonym of *Schistometra conoides* (Bloch, 1782).

We will now consider the remaining species.

ANOPLOTAENIA DASYURI, Beddard, 1911

Synonym :—

Oochoristica dasyuri (Beddard, 1911), Meggitt, 1924.

Host :—*Sarcophilus satanicus*, Thomas. Locality :—Tasmania (Lond. Zoo.).

Of this worm we were able to examine two entire specimens and a few fragments. The length of the largest specimen is 23 mm., and the greatest width is 1 mm. There are altogether about thirty-one segments ; these are at first broader than long ; they then become square, and finally longer than broad, the last segments measuring 2·7 mm. in length, and 1·4 mm. in width.

The *scolex* is very typical, and measures about 0·86 mm. in diameter ; it is provided with four very large suckers, oval in shape

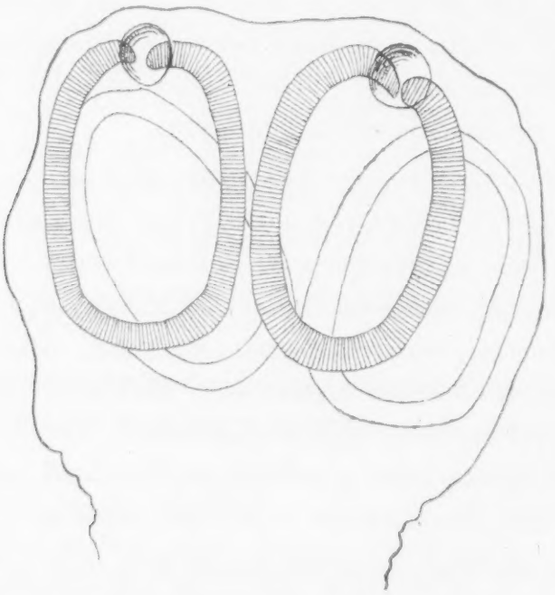

Fig. 1. The scolex of *A. dasyuri*, Beddard.

(fig. 1) measuring 0·48 : 0·29 mm. Neither in whole mounts nor in sections is there any trace of a rostellum. On page 1005, Beddard (1911b) states that ' there is in the same way a kind of hint of a

commencing pseudo-scolex.' What Beddard saw, and interpreted in the above manner, is nothing less than the folds arising from the contraction of the first segments of the strobila.

The cuticle is 3·8μ thick; there are no calcareous corpuscles. The musculature is fairly well-developed. The longitudinal musculature consists of two layers, one outer layer of stout fibres irregularly dispersed throughout the cortical parenchyma, and reaching almost as far as the cuticula (fig. 2), and one inner layer

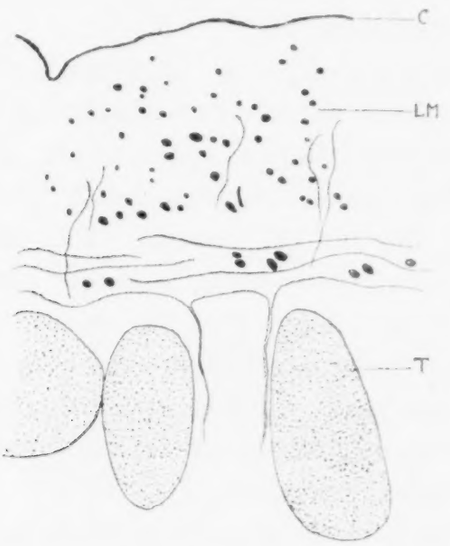

FIG. 2. *A. dasyuri*. A portion of a transverse section. C.—cuticula; L.M.—longitudinal musculature; T.—testes.

of very stout fibres, usually found in pairs, but not forming bundles as described and figured by Beddard (*loc. cit.*, p. 1005, fig. 209). We have, however, occasionally found the latter disposition in the last segments of the strobila. The transverse muscles are but feebly developed; they roughly form two layers separating the inner longitudinal muscle layer from the outer layer on one side, and from the medullary parenchyma on the other. Dorso-ventral fibres are fairly numerous throughout the strobila.

The two longitudinal *nerves* are very much compressed laterally, and measure 15·5μ, the greatest diameter being dorso-ventrally.

The *excretory system* is well developed, and consists of the usual four longitudinal vessels. The ventral vessels are about 0·03 mm. in diameter on transverse sections, and present a very typical aspect in the end segments. At the point where the transverse vessel branches

off, the ventral vessel suddenly swells out, forming a kind of reservoir. This disposition is fairly common among Cestodes, and is also found in *Hemiparonia cacatuae* (Maplestone, 1922), described below. The dorsal excretory vessels are not more than 0·009 mm. in diameter, and are situated dorsally and slightly internal to the ventral vessels. The genital ducts pass between the dorsal and ventral excretory vessels. We have been unable to determine with certainty the position of the longitudinal nerve stem with regard to the genital ducts; it seems, however, to lie ventral to the latter. The genital pores are irregularly alternate.

Genitalia. The *testes* are very numerous, about 300 or more, and lie in two to three dorso-ventral layers anterior to the cirrus pouch and to the coils of the vas deferens (fig. 3, B). There are no testes to be found dorsal to the cirrus pouch, to the coils of the vas deferens, and to the female gonads; the latter are, however, surrounded by testes, there being a single row posterior to the vitelline gland (fig. 5). The testes are usually close together, and are ovoid in shape, the greatest diameter being dorso-ventrally.

The *vas deferens* takes up an extraordinary amount of room, pushing aside the testes and the uterus, and occupying most of the available dorso-ventral space.

After forming an intricate mass of coils the vas deferens penetrates into the cirrus pouch. The latter has been described at much length by Beddard, who has, however, failed to interpret this organ correctly, and has caused much confusion by trying to distinguish within the cirrus pouch a vas deferens, a cirrus and a penis. The cirrus pouch is almost spherical in shape, usually broader than long. It measures 0·19 mm. in length, and 0·21 mm. in diameter. Its walls are fairly muscular, and are 5μ thick, being chiefly constituted of longitudinal fibres. Within the pouch the vas deferens forms several loose coils, which Beddard (*loc. cit.*, p. 1015) has interpreted as the cirrus. The *cirrus* is 0·21 mm. long and 0·03 mm. in diameter; it is unarmed and somewhat swollen towards its extremity, and possesses a *terminal* pore. Beddard's drawing and description of a lateral pore are, of course, due to oblique sections. The cirrus is covered with a fairly thick cuticle, the latter being usually thicker towards the base of the cirrus. Within the cirrus pouch are to be found numerous muscle fibres acting, no doubt, as *retractores*

cirri. There are also numerous small cells with large nuclei, which we believe to be the myoblasts of the above muscles. The cirrus pouch opens into a genital atrium, which is extremely characteristic, reminding one in some ways of a similar

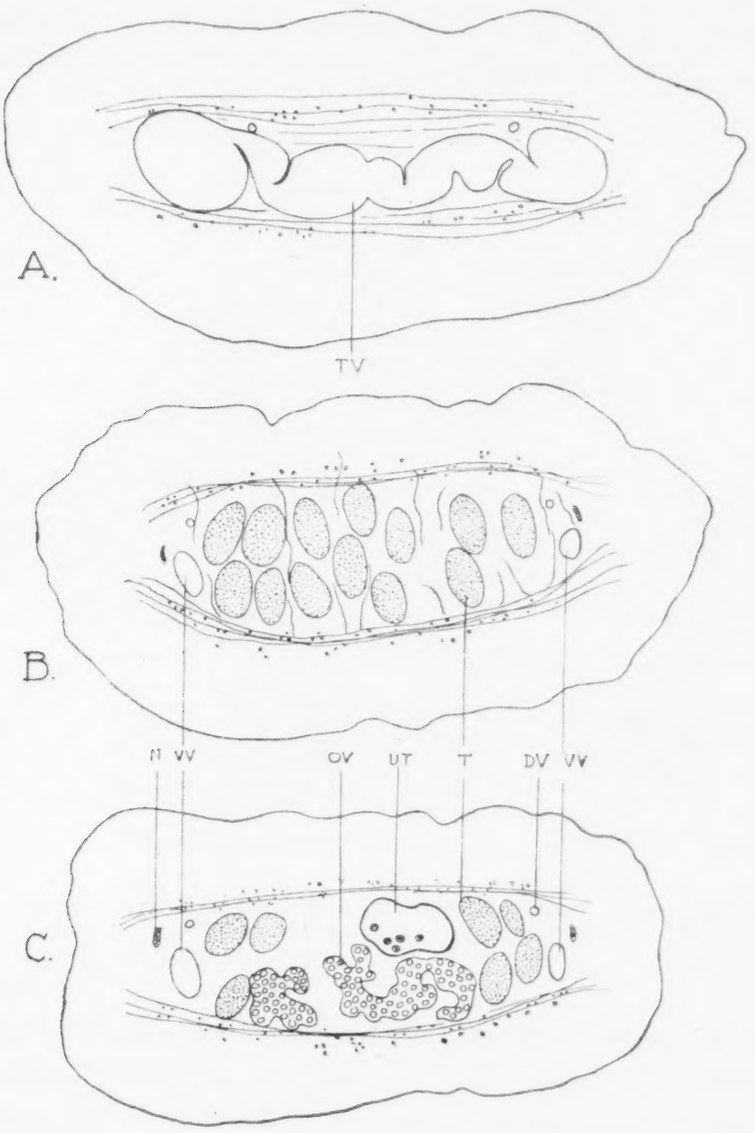

FIG. 3. *A. dasyuri*. Transverse sections: A, between two segments; B, anterior to the cirrus pouch; C, posterior to the cirrus pouch. D.V.—Dorsal excretory vessel; N.—nerve; OV.—ovary; T.—testes; T.V.—transverse vessel; V.V.—ventral excretory vessel; UT.—uterus.

structure found in *Tetrabothrius* spp. The genital atrium may be divided into two regions and not into three or four, as Beddard describes. Immediately next to the cirrus pouch we find a tremendous

sphincter muscle 0·12 mm. in diameter, and 0·1 mm. thick on transverse sections. Curiously enough, this sphincter is pierced laterally to permit the vagina to open into the atrium. Beyond the sphincter we find a second region or atrium proper, the walls of which are provided with numerous radiating muscle fibres, the latter belonging partly to the system of transverse muscles (fig. 4, A and B). It would seem that this complicated genital atrium would

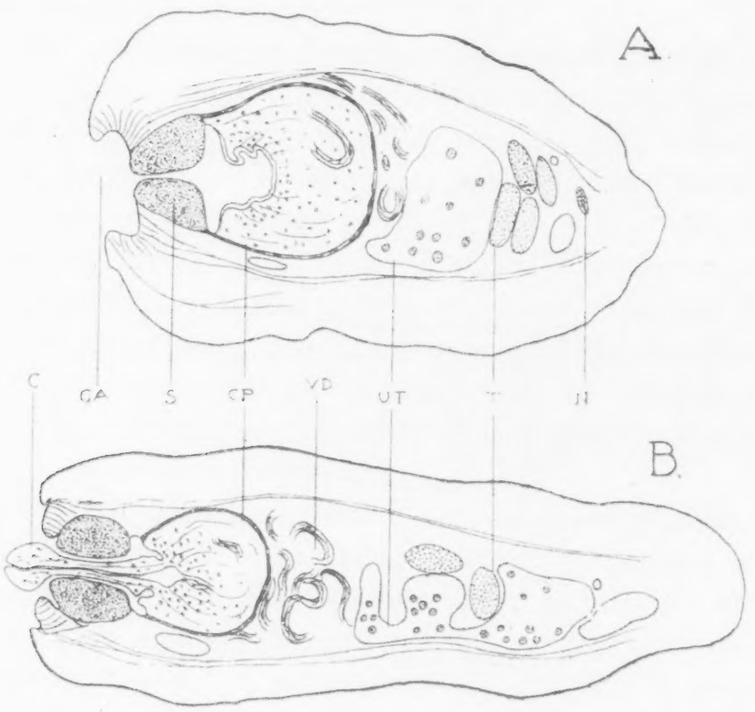

FIG. 4. *A. dasyuri*. A and B, transverse sections passing through the cirrus pouch. C.—cirrus; C.P.—cirrus pouch; G.A.—genital atrium; N.—nerve; S.—sphincter; T.—testes; UT.—uterus; V.D.—Vas deferens.

function as follows. When the cirrus is to be protruded, the transverse muscles contract, thus opening the anterior chamber of the atrium, and at the same time pressing on the walls of the cirrus pouch, causing the cirrus to evaginate; cross-fertilisation is thus made possible. When, however, the sphincter is closed, then self-fertilization is rendered possible, the seminal fluid being expelled through the contractions of the muscular walls of the cirrus pouch. The *vagina*, as we have already mentioned, perforates the sphincter laterally, and passes posterior to the cirrus pouch after forming

a sudden curve, almost at right angles (fig. 5). In its distal portion the vagina forms a distinct and fairly large receptaculum seminis. The *ovary* consists of two wings, of which the poral one is slightly smaller than the aporal one. These wings are made up of fairly numerous and somewhat compressed lobes and remind one strongly of the ovaries of *Taenia* spp. The *vitelline gland* is fairly compact, situated posterior to the ovary, and not extending laterally beyond the latter. The shell gland is well developed. The *uterus* appears very soon and as in *Taenia* spp., consists of a median stem, the

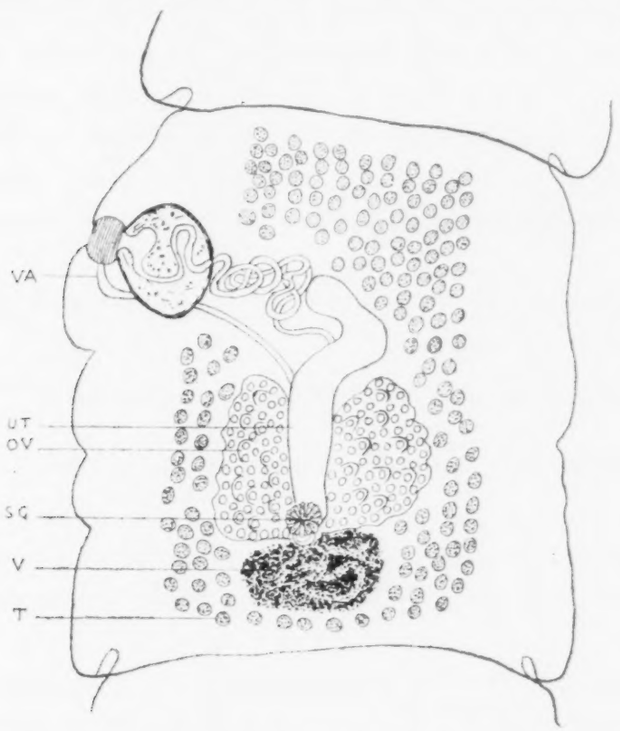

FIG. 5. *A. dasyuri.* A mature proglottid. OV.—ovary; S.G.—shell gland; T.—testes; UT.—uterus; V.—vitelline gland; VA.—vagina.

anterior portion of which is pushed aside by the coils of the vas deferens, thus forming a very characteristic kink. The uterus soon begins to branch out laterally until it fills almost the entire segment. On no occasion was the uterus observed to be reticular, neither were ova found embedded in the parenchyma, both these observations being due to errors of interpretation. The gravid uterus presents a very characteristic aspect, there being always more diverticula on the aporal than on the poral side (fig. 6). The *ova* are thin-shelled and measure 27 : 19μ.

As can be gathered from the above description, the genus *Anoplotaenia*, Beddard, 1911, is entirely justified, although to our mind its systematic position is not correct.

Beddard, after a somewhat lengthy discussion, places his genus in the sub-family ANOPLOCEPHALINAE because the head is unarmed, and because the host is a Marsupial.

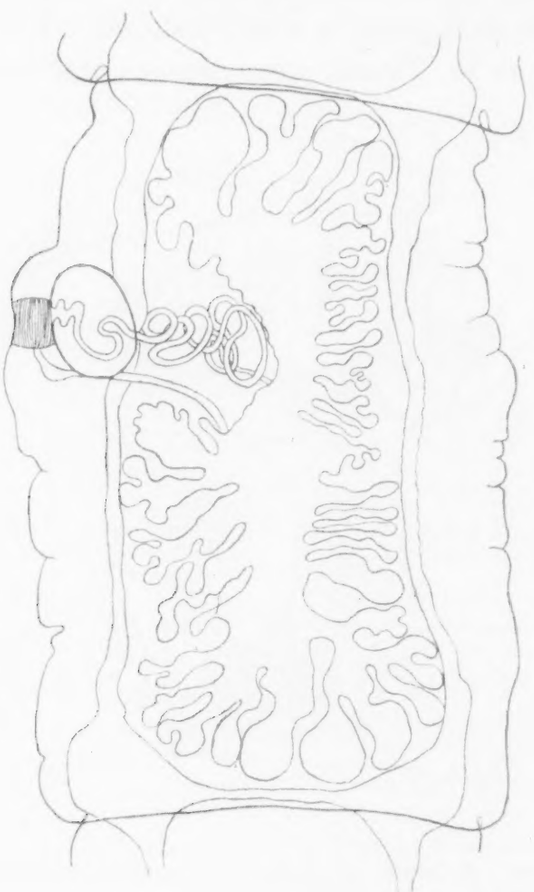

FIG. 6. *A. dasyuri*. A gravid segment showing the structure of the uterus.

Meggitt (1924a) basing his argument on Beddard's error of interpretation, considers the genus *Anoplotaenia* as a synonym of *Oochoristica*, because the uterus dissolves and the ova are scattered in the parenchyma, and he lists it accordingly. To our mind the presence of an unarmed scolex is not necessarily a reason for including this genus in the ANOPLOCEPHALIDAE. Several families of *Cyclophyllidea* contain genera with unarmed scolices, the same genera being also found to possess species with armed scolices: for instance,

Hymenolepis and *Taenia s. str.*, not to mention *Anonchotaenia*, *Rhabdometra*, *Octopetalum*, etc. On the other hand, the uterus of *Anoplotaenia*, as Beddard himself states, is very similar to that of *Taenia s. str.*

If we now take the above characters into consideration, keeping in mind the general anatomy, we can but place the genus *Anoplotaenia* in the family TAENIIDAE, Perrier e. p., and re-define it as follows :—

TAENIIDAE *of small size. Head unarmed, suckers large. Genital pores irregularly alternating. Genital ducts pass between the excretory vessels and dorsal (?) to the nerve. Testes form a single field interrupted dorsal to the coils of the vas deferens and to the ovary and vitelline gland. A single row of testes posterior to the latter. Cirrus pouch spherical, opening into a highly differentiated genital atrium provided with an exceedingly powerful sphincter. The latter is perforated laterally by the vagina. Uterus a median stem with numerous lateral diverticula.*

Adult in *Marsupials*. Type : *Anoplotaenia dasyuri*, Beddard, 1911.

DASYUROTAENIA ROBUSTA, Beddard, 1912

Host :—*Sarcophilus satanicus*, Thomas. Locality :—Tasmania (Lond. Zoo).

This second interesting genus was also obtained by Beddard from a Tasmanian Devil. Unfortunately we have only been able to examine a few fragments of this worm.

The greatest length according to Beddard (1912b) is 31 mm. and the greatest width 9 mm. The *scolex* 3·5 mm. in diameter bears four suckers, each of which measures 0·35 mm. in diameter. The fragments to hand, and also the material examined by Beddard, judging from his drawings, are extraordinarily contracted. This will serve to explain certain of the errors committed by Beddard.

The *cuticle* is exceedingly thick, and measures as much as 11·4μ. There are no calcareous corpuscles to be found.

The *musculature* is extremely well developed (fig. 7). Immediately beneath the cuticula we find a layer of irregularly disposed muscle fibres ; these are very stout and show a tendency to form bundles of about four fibres each. Beneath this layer of longitudinal muscles are to be found several fibres of transverse muscles. Beneath

these again we find a second layer of longitudinal muscles now definitely grouped in bundles containing about fifteen fibres. We next find a second layer of transverse muscles, beneath which lies a third layer of longitudinal muscles forming bundles containing about fifty fibres each. This layer is separated from the next by a third layer of transverse fibres. The fourth layer of longitudinal muscles consists of bundles containing about thirty fibres each. We then find a fourth layer of transverse fibres, beneath which lies a fifth layer of longitudinal muscle bundles containing about twenty fibres each. Finally we have a fifth layer of transverse fibres.

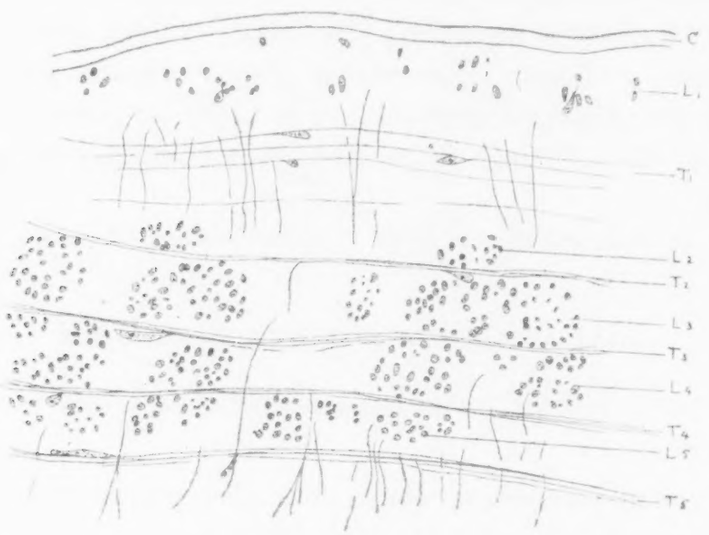

FIG. 7. *D. robusta*. A portion of a transverse section. C.—cuticula: L1-L5.—Longitudinal musculature; T1-T5.—transverse musculature.

Dorso-ventral fibres are very numerous. It is interesting to note the very numerous and exceedingly distinct myoblasts, the latter being found in all three of the muscular systems. This exceedingly powerful musculature reminds one of that of *Cotungia* spp. and also to a certain extent of that of the ACOLEIDAE.

The *excretory* system also presents a very interesting disposition, and seems to have given Beddard much trouble. The most striking character of this system is the truly extraordinary development of the two ventral excretory vessels. The latter are about 0·69 mm. in diameter, and form two exceedingly large coils in the lateral fields of the proglottides. Owing to extreme contraction of the worm, we find these coils touching one another, with the result that on

sections we find what Beddard describes as membranes and valvules, and which are caused by the sections passing somewhat obliquely through two consecutive coils. In the same way the genital ducts do not *pierce* the ventral vessel, but pass between two coils. We have endeavoured to figure this diagrammatically in fig. 8. The latter

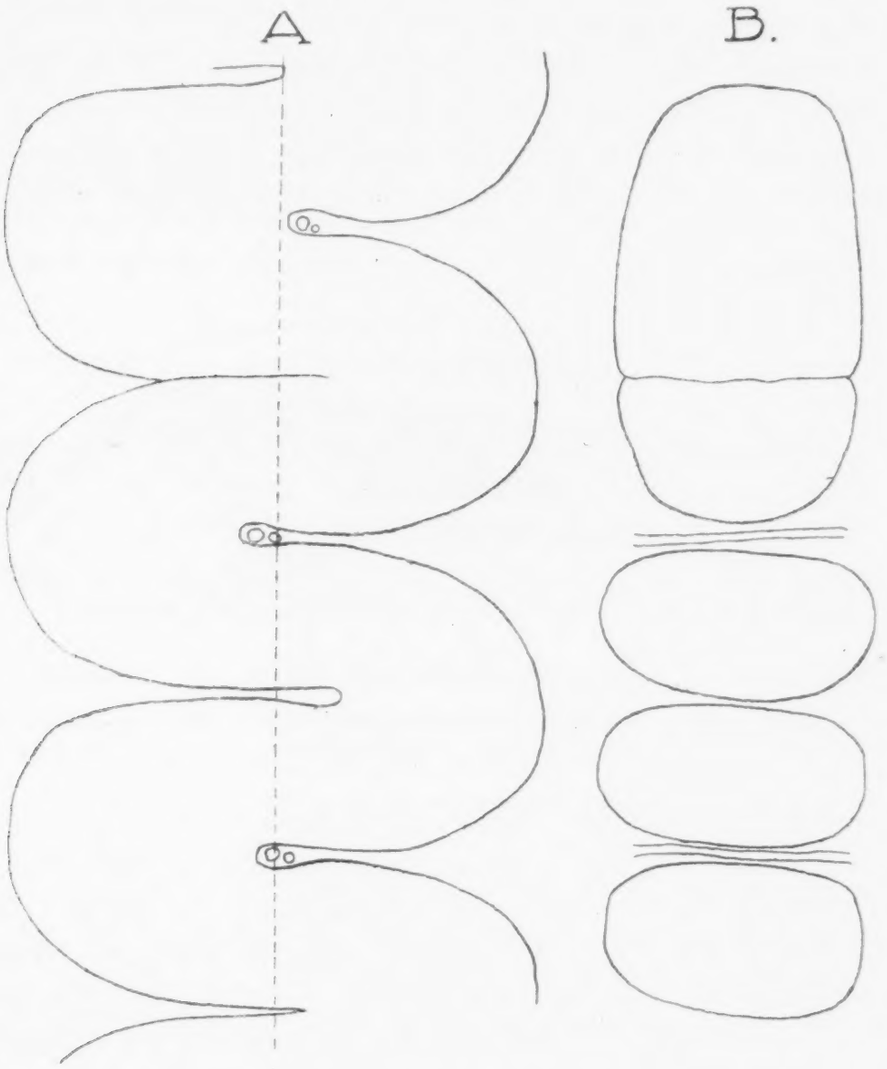

FIG. 8. *D. robusta.* Diagram of the ventral excretory vessel: A, sagittal view; B, section along the dotted line viewed horizontally.

represents a ventral vessel coiled dorso-ventrally; in reality the coils are spiral, and next to it is a section passing along the dotted line. As Beddard rightly remarks, there are no transverse vessels to be seen, although perhaps in less contracted specimens such

a structure might be seen, and might have been obliterated owing to the extraordinary contraction of the worm. The dorsal vessels are about 0·007 mm. in diameter, i.e., about a hundred times smaller than the ventral vessels. They are also very much coiled and are exceedingly difficult to make out.

The two lateral *nerve stems* are compressed laterally and measure on transverse section 76 by 19μ.

Genitalia. The genital pores are unilateral, and the genital ducts pass between the excretory vessels, and ventral to the nerve.

The *testes* are fairly numerous, about 250, and are inclined to be dorsal (fig. 9). They occupy three to four dorso-ventral layers in the

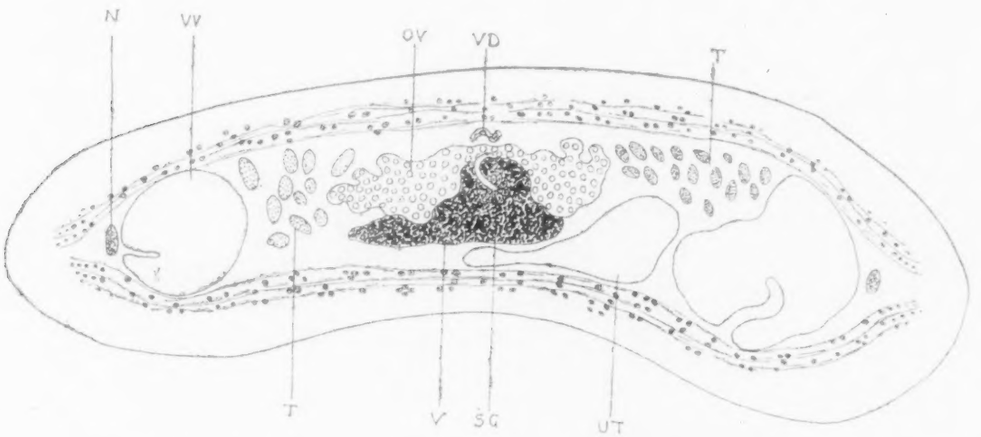

FIG. 9. *D. robusta.* Transverse section. N.—nerve; OV.—ovary; S.G.—shell gland; T.—testes; UT.—uterus; V.—vitelline gland; V.D.—vas deferens; V.V.—ventral excretory vessel.

lateral fields, and only a single dorsal layer in the region of the female genitalia. This layer is, however, soon pushed aside by the growing female organs and especially by the uterus. When examined in horizontal sections (fig. 10), the testes are flattened antero-posteriorly, owing to contraction. Laterally the testes extend as far as the ventral excretory vessels. The *vas deferens* is extremely coiled, but straightens out suddenly when passing between the excretory vessels. We have not noticed the glands surrounding the male duct and described by Beddard as 'interstitial prostatic cells.' The cirrus pouch is an elongate pear-shaped organ 0·34 mm. long and 0·25 mm. at its greatest diameter. The walls are fairly muscular and measure 8μ in thickness. Immediately on entering the cirrus pouch the vas deferens forms several coils much distended with

spermatozoa, and probably replacing functionally an internal vesicula seminis. The *cirrus* is 0·19 mm. long and 0·015 mm. in diameter. Although none of the cirri were evaginated, we have been unable to observe on them any small spines such as Beddard describes on page 693. The *vagina* opens into a small genital atrium posterior to the cirrus pouch ; it is thick-walled in the first part of its course and runs almost in a straight line towards the centre of the segment, where it forms a large receptaculum seminis. We have noticed an interesting and somewhat problematic structure situated on the course of the vagina and just before the latter enters the receptaculum seminis. The vagina suddenly increases in diameter and becomes thick-walled resembling very much an oötype. This structure contains circular muscle fibres, and probably acts as

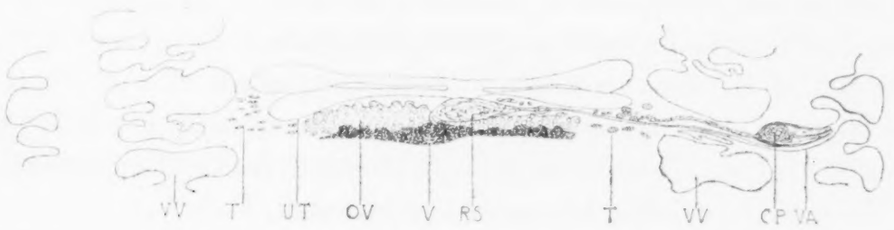

FIG. 10. *D. robusta*. Horizontal section. C.P.—cirrus pouch ; OV.—ovary ; R.S.—receptaculum seminis ; T.—testes ; UT.—uterus ; V.—vitelline gland ; VA.—vagina ; V.V.—ventral excretory vessel.

a sphincter to close the receptaculum seminis once the latter is filled with spermatozoa. The *ovary* is only slightly lobed and forms two wings, of which the poral one is slightly smaller than the aporal one. The *vitelline gland* is situated posterior and slightly ventrally to the ovary, extending laterally as far as the latter. A distinct shell-gland is also to be found. The *uterus* is not as Beddard states 'a large cavity extending right across the segment,' but presents the typical structure met with in the genus *Taenia*, i.e., it possesses a median stem with two or more lateral diverticula. These latter are club-shaped when distended with ova. With regard to the 'uterine pore' described in a later paper (1915), we cannot but interpret this as an artefact due to the sections not being in an horizontal plane.

The ova measure 19 by 15μ, and are provided with a thin shell.

It is obvious that the above description is far from being complete as no young segments, and especially no scolex, have been examined, the description being entirely based on gravid or mature segments.

We have attempted to clear up certain obscure anatomical details, but remain totally in the dark with regard to the scolex.

Beddard's description of a globular scolex with two external and two internal suckers depicts an entirely new arrangement, and suggests that the author has misinterpreted his sections. We cannot, of course, say that the arrangement described by Beddard is impossible without having examined the material; we may, however, formulate an hypothesis based on the general structure of the worm, and on Beddard's drawings of the head. The hooks figured on page 682, text-fig. 96, are typical hollow hooks as found on the rostellum of many species of Cestodes, and especially in the family TAENIIDAE. The anatomy, also, is that of a member of this family; we are, therefore, led to conclude that the '*two inner suckers armed with hooks*' represent a rostellum! The fact is much more apparent if the figure is held upside down. Hence we conclude that the genus *Dasyurotaenia* should be maintained, and placed in the family TAENIIDAE and not in the ANOPLOCEPHALIDAE as Meggitt (1924b) has done, basing his classification on Beddard's description.

We would re-define this genus as follows:

TAENIIDAE *of small size, with an exceedingly well-developed muscular system. Ventral excretory vessels hypertrophied, a transverse vessel being absent* (?) *Scolex provided with four small suckers and a rostellum armed with a double* (?) *crown of hooks. Genital pores unilateral; genital ducts passing between the excretory vessels and ventral to nerve. Large receptaculum seminis present. Uterus with a short median stem and very long, club-shaped, lateral diverticula.*

Adult in Marsupials. Type: *Dasyurotaenia robusta*, Beddard, 1912.

MONOECOCESTUS ERETHIZONTIS, Beddard, 1914

Host: *Erethizon dorsatum* L. Locality: N. America (Lond. Zoo).

We have been able to examine two entire specimens of this worm; all our observations being made on total mounts, the latter being remarkably clear, as the material is rather macerated.

A careful examination of our preparations has shown us that the excretory vessels show distinct anastomoses such as are always found in the genus *Schizotaenia*. Beddard describes at much

length (p. 1048) the presence in certain segments of a rudimentary vagina, and based on this character, he places his genus in the ACOLEIDAE! One of the characteristics, however, of the ANOPLOCEPHALINAE, with the exception of *Aporina*, is the possession of a vagina which soon atrophies and disappears as the segments grow older. Beddard has himself described this particularity, but he does not seem to have realised its importance.

The rudimentary vagina of the ACOLEIDAE is of a totally different type, the vaginal *pore* being absent.

We have found the cirri to be covered with small spines, and what is more, we have found that the material examined contains *two* different species! Our observations lead us to conclude that what Beddard has described as *Monoecocestus erethizontis*, gen. et sp.n. is nothing else than *Schizotaenia americana* (Stiles, 1895), and *Schizotaenia variabilis*, Douthitt, 1915. Beddard's paper is dated 1914, and Douthitt's 1915. Although the latter author's description of *S. variabilis* is excellent, we must comply with the rules of priority. This species must, therefore, be named *S. erethizontis* (Beddard, 1914), syn. *S. variabilis*, Douthitt, 1915.

In the following table we have endeavoured to place all the species of the genus *Schizotaenia*, Janicki, 1904, giving the differential characters of each species. It will be noticed that we retain the specific name *americana* for the species described by Stiles (1896). Douthitt (1915) has definitely shown the existence of two species of Tapeworms in the American Porcupine, neither of which can be identified with *T. laticephala*, Leidy, 1855. As the types of the latter have been lost (*vide* Stiles, *loc.cit.*, p. 165), it seems undesirable to maintain this name. We, therefore, propose to consider *T. laticephala*, Leidy, as a *nomen nudum*. We have removed from the genus *Schizotaenia* the species *S. cacatuae*, Maplestone, 1922, as this species is the type of a new genus to be described below. In a previous paper (1923), we removed to the genus *Anoplocephala* the species *S. latissima* (Deiner, 1912) and *S. gigantea* (Peters, 1856). We are almost inclined to include in the genus *Schizotaenia* the species actually known as *Oochoristica didelphydis* (Rudolphi, 1810) from *Marmosa murina*, L. It will be remembered (*vide* Janicki, 1906) that only fragments of this species exist, egg-capsules have not been found, and the scolex is unknown. On the other hand, the

vagina lies anterior to the cirrus pouch, and the ovary and the uterus are just at the stage where it is almost impossible to distinguish the one from the other, especially in macerated material. These last two factors seem to show a distinct relationship to the genus *Schizotaenia*. We prefer, however, to leave this species where it is for the present, and await a further supply of material in order to be able to study it further.

TABLE I

Species	Author	Year	Length	Width	Diameter of scolex	Dimensions of cirrus pouch	Number of testes	Size of ova	Host	Distr.
S. decrescens ...	(Diesing)	1856	mm. 296	mm. 5	mm. ?	mm. 0·67 : 0·23	?	μ ?	*Tayassus tajacu, T. albirostris*	Braz.
S. bagmanni ...	Janicki	1904	145	5·8	1·9	0·63 : 0·2	120–140	57	*Hydrochoerus capybara*	Braz.
S. americana ...	(Stiles)	1895	33	6	0·6	0·5–0·63 : 0·19–0·21	70	55–61	*Erethizon dorsatum, E. epixanthum*	U.S. Can.
S. sigmodontis ...	Chandler and Suttles	1922	30–50	2·5–3·5	0·30–0·45	0·6 : 0·10	70	47–53	*Sigmodon hispidus*	Tex.
S. anoplocephaloides	Douthitt	1915	30–33	1·7–2	0·39	0·14 : 0·085	70–110	30–40	*Geomys breviceps*	U.S.
S. erethizontis ...	(Beddard)	1914	25	8·5	0·88	0·40–0·53 : 0·14–0·19	70–110	12–14	*Erethizon dorsatum*	U.S.

It is interesting to note that the genus *Schizotaenia* is entirely confined for the present, to the New World, where it is found in *Rodentia* and *Suidae*. A point which may be of some importance and which appears very interesting is raised by Scharff (1911). Speaking of the Canadian Tree Porcupine, the author says :

'Yet the species had already come into existence when the sabre-tooth tiger and peculiar kinds of peccaries haunted the forests of Arkansas, for its remains have been found together with these extinct creatures in the Conrad fissure.'

Actually the peccaries have retreated into South America, and the Canadian Tree Porcupine has retreated further north ; both groups, however, harbour the same genus of Cestode parasites. May we

take this as an indication that the genus *Schizotaenia* came into existence during the late Tertiary times, about the Pliocene Period, and has existed ever since ? The collection of further data will show if such an hypothesis is liable to lead to interesting results, or is to be abandoned.

THYSANOTAENIA LEMURIS, Beddard, 1911

Host :—*Lemur macaco*, L. Locality :—Madagascar (Lond. Zoo).

We have only been able to examine a few gravid and much macerated fragments of this worm, and are totally unable to add anything to Beddard's description except that a distinct dorsal excretory vessel is present in all our sections. This genus is, however, not a synonym of *Inermicapsifer*, Janicki, 1910. From the description of the genital organs, and from the aspect of the egg-capsules, we are almost inclined to refer this genus to *Raillietina*, Fuhrmann, 1920, and to the sub-genus *Ransomia*. We would do this in spite of the fact that Beddard states that the scolex is unarmed. All workers who have had to deal with this group know how difficult it is at times to perceive the tiny hooks on the rostellum; especially when the latter is retracted. For the present, however, and until more material has been examined, we retain the genus *Thysanotaenia*, Beddard, 1911, with the type species *T. lemuris*, Beddard, 1911, and place it in the sub-family LINSTOWINAE.

HEMIPARONIA CACATUAE (Maplestone, 1922), n.gen.

Synonym :—

Schizotaenia cacatuae, Maplestone, 1922.

Host :—*Cacatua galerita*, Lath. Locality : North Queensland.

As we have already mentioned above, this species was placed by Maplestone in the genus *Schizotaenia*. Its curiously aberrant anatomy, however, led us to suppose that this might be the type of a new genus. Thanks to the kindness of Professor Warrington Yorke, we have been able to examine the type and also the type material deposited in the collection of the School, and have been able to confirm our first opinion. We also express our sincerest thanks to Dr. Southwell for the loan of this valuable material.

Certain points in Maplestone's description are not very clear; we will therefore briefly redescribe the anatomy.

The *cuticula* is 4μ thick, and beneath this lies the internal longitudinal *musculature*. The latter consists of three layers of stout bundles. As the material is considerably macerated, it is very difficult at times to make out the three layers; these exist, however, throughout the entire strobila. Transverse muscles are hardly developed, whereas the dorso-ventral muscles are very numerous. The cortical parenchyma contains numerous small calcareous corpuscles measuring $7{\cdot}6 : 5\mu$.

The two longitudinal nerve stems lie lateral to the dorsal excretory vessels.

Genitalia. Maplestone estimates the number of *testes* at 100; we believe, however, that this number is too small. The testes lie in two and sometimes three dorso-ventral layers (fig. 11). We should

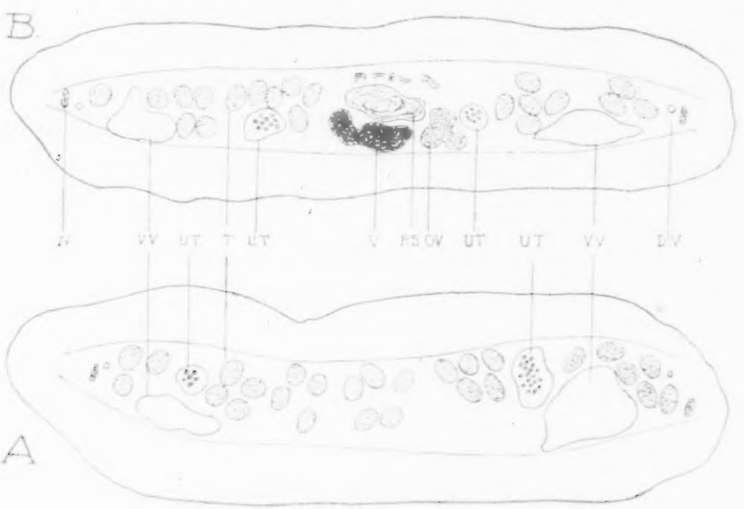

FIG. 11. *H. cacatuae*. Transverse sections: A, through the anterior region of the segment; B, through the posterior region of the segment. D.V.—Dorsal excretory vessel; N.—nerve; OV.—ovary; R.S.—receptaculum seminis; T.—testes; UT.—uterus; V.—vitelline gland; V.V.—ventral excretory vessel.

say that there appear to be about 200 testes. The latter are spherical in the lateral fields, and measure 0·042 mm. in diameter. Towards the centre of the segment they are generally so crowded together that they become egg-shaped, the greatest diameter being dorso-ventral. Laterally the testes pass beyond the ventral

excretory vessels. The vasa efferentia form a distinct and very complicated network, a portion of which is drawn in fig. 12. The

FIG. 12. *H. cacatuae.* Portion of the network formed by the vasa efferentia.

vas deferens soon becomes swollen with spermatozoa. Within the cirrus pouch it forms a few coils also very much distended with spermatozoa; it then enters the cirrus. The latter is covered with minute spines. The *vagina* opens slightly anterior and ventral to the cirrus pouch. Passing ventrally to the vas deferens it forms a large receptaculum seminis situated dorsally (fig. 13). The vagina

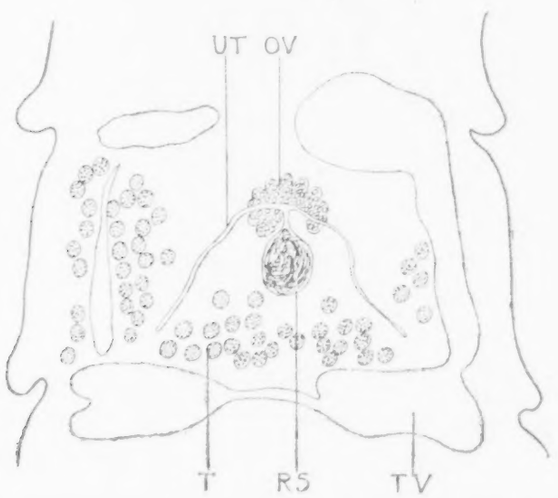

FIG. 13. *H. cacatuae.* A horizontal section through a young proglottid. OV.—ovary; R.S.—receptaculum seminis; T.—testes; T.V.—transverse excretory vessel; UT.—uterus.

very soon becomes so distended with spermatozoa that it is impossible to distinguish it from the receptaculum seminis (fig. 14). The *ovary* and *vitelline gland* are situated one behind the other; the former, fan-shaped, is made up of several lobes, and is situated ventrally to the latter, which is only slightly lobed. This can be

seen in transverse sections passing through this region (fig. 11). We find a slightly different arrangement of the female ducts from that described by Maplestone. The oviduct which is surrounded by numerous glands, receives first of all the duct from the receptaculum seminis, then and on the same side, the vitelline duct, and only then does the shell gland surround the oviduct, the latter passing into the uterus. The *uterus* constitutes the chief character of our new genus. In *anlage* (fig. 15) it appears as a fine horseshoe-shaped tube

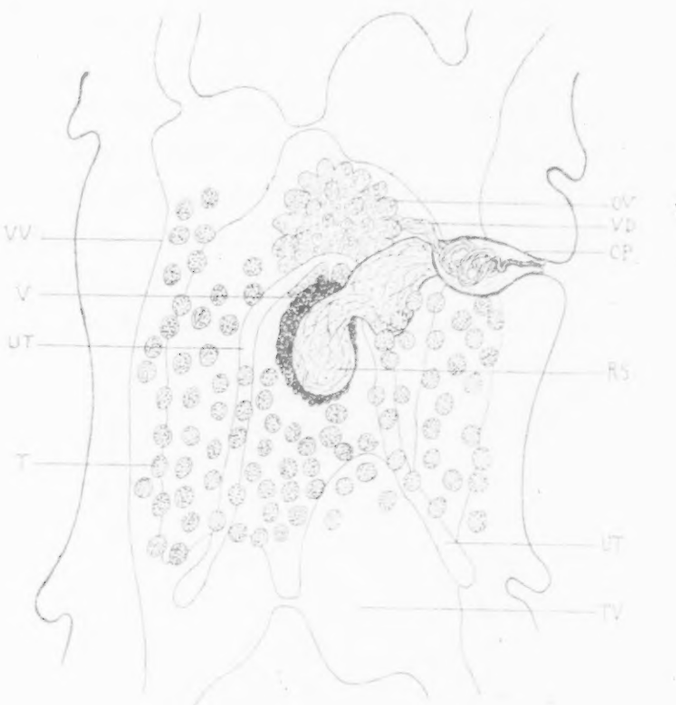

FIG. 14. *H. cacatuae*. A mature segment. C.P.—cirrus pouch; OV.—ovary; R.S.—receptaculum seminis; T.—testes; T.V.—transverse excretory vessel; UT.—uterus; V.—vitelline gland; V.D.—vas deferens; V.V.—ventral excretory vessel.

passing between the ovary and the vitelline gland, and lying in the centre of the segment. Very soon, however, the uterus increases in diameter, and forms several diverticula. In the gravid uterus the two extremities of the horseshoe never fuse together.

This extremely interesting genus from an Australian parrot bears an extraordinary resemblance to the genera *Paronia* Diamare, and *Moniezioides* Fuhrmann (*vide* Fuhrmann, 1918), both of which are also found in Australian parrots. The only difference is that these

two genera possess double genital pores, and a double genital apparatus.

Our new genus possesses unilateral, dextral genital pores, and the vagina lies ventral to the cirrus pouch. We would obtain the same disposition if we were to cut a species of *Paronia* into half, and considered the right half only; this has led us to propose the name *Hemiparonia*, n.gen., and we define it as follows:—

FIG. 15. *H. cacatuae*. The relationship of the female ducts. OV.—ovary; R.S.—receptaculum seminis; S.G.—shell gland; UT.—uterus; V.—vitelline gland.

ANOPLOCEPHALINAE *of moderate size. Genital pores unilateral and dextral. A single set of reproductive organs in each segment. Vagina ventral to cirrus pouch. Genital ducts dorsal to excretory ducts and nerve. Testes a single dorsal field extending laterally beyond the ventral excretory vessel. Large receptaculum seminis present. Ovary and vitelline gland in centre of segment, former anterior to latter. Uterus horseshoe-shaped, later forming diverticula; the two extremities never fuse together. Ova without (?) piriform apparatus.*

Adult in Birds. Type: *Hemiparonia cacatuae* (Maplestone, 1922).

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ON THE VALUE OF THE ESTIMATION OF THE IONIC CALCIUM OF THE SERUM IN THE DIAGNOSIS OF, AND AS A GAUGE OF PROGRESS IN SPRUE

BY

H. HAROLD SCOTT, M.D., M.R.C.P. (London), D.P.H., D.T.M. & H. (Camb.), F.R.S. (Ed.)

MILNER RESEARCH FELLOW IN COMPARATIVE PATHOLOGY, LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

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This contribution on the subject of sprue has a two-fold purpose. Firstly, to show the value of the estimation of the 'Ionic' calcium as a diagnostic criterion; secondly, to demonstrate that the same test, when repeated at intervals during the course of the disease, affords a valuable, in fact the most reliable gauge of progress.

Vines (1921) has shown that the calcium in the plasma normally exists in two forms. Of the total (10—11 mgm. per 100 c.c.) some 60 per cent. is readily precipitable by its chemical equivalent of ammonium oxalate solution, whereas the remaining 40 per cent., being 'bound,' probably with a lipoid complex, requires nearly three times its corresponding chemical equivalent. This latter, being closely concerned with the clotting of blood, is designated 'coagulative' or 'combined' calcium. When coagulation occurs, the latter becomes converted into the former, so that normal serum, as distinguished from plasma, contains all the calcium in the readily precipitable 'free' or 'ionic' form. If, on analysis of the serum, the total calcium is found to be normal in amount, whereas the ionic calcium is reduced, some of the calcium must have again become bound, and it is believed that the parathyroid glands, if acting normally, prevent this change.

An examination of this question, which is dealt with in Vines's work—*The Parathyroids in Relation to Disease* (1924), appears to show that 'there is no reason for assigning the control of calcium metabolism to any other endocrine gland' nor can any other gland

'wholly or partially restore the disorders of calcium metabolism consequent on parathyroid failure or removal.'

It is clear that there may be two types of calcium deficiency, according as the total is decreased owing to there being an excessive excretion and a resultant calcium starvation of the tissues, or, the total being normal, the active or ionic calcium is deficient. In sprue the latter is the case. In very severe forms of the disease the total may be a little diminished, but never, as far as my observations go, to any marked extent. The error, therefore, would appear to be due, not to faulty absorption from the alimentary canal, but either to failure on the part of the tissues to use this calcium, or to errors in the regulation of calcium excretion—'a lowering of the threshold of excretion.' It is believed that in either case the underlying factor is a circulating toxin, which brings about the results found in sprue, by effecting a combination with the calcium of the blood or, perhaps, by damaging or interfering with the function of the parathyroids.

Further, toxaemias are said to stimulate thyroid function, and the antagonistic action of this gland to that of the parathyroid is well exemplified in sprue. If an impure preparation of parathyroid is used in treatment, that is, one containing any thyroid, or if, when progress is being made, parathyroid is stopped and thyroid given in its place, a return of the symptoms can be readily induced.

Dealing first with the value of the calcium estimation in diagnosis, it is particularly in diarrhoeic conditions that a reliable test is needed. We need not, therefore, discuss the diagnosis of diseases which are distinguishable on other clinical grounds. In pellagra, for example, there may be at times watery and offensive stools, with gastric and intestinal flatulence. There may also be a diffuse inflammation of the mouth. But these symptoms are clinically distinguishable from those in sprue, and there is nearly always a history of severe, bilaterally symmetrical 'sunburns' in the spring, and evidence of pigmentation and roughness (pell' agra) on the back of the hands, the face and neck, to confirm the diagnosis.

In an article published recently in the *Journal of the American Medical Association* the writers Bastedo and Famulener (1923) discuss in full the means available for the diagnosis of sprue, cultural examination of bacteria, yeasts and so forth, and end by stating

'In sprue we have a disease for which no fully reliable laboratory criteria have been established.' I hope to demonstrate in the present paper that this no longer holds good.

In sprue it is the intestinal condition which first shows itself in the vast majority of cases—the early morning call to stool, with increasing bulk and frequency of pultaceous, frothy, fermenting motions. Intestinal disturbances are frequent in the tropics, and it is all-essential that treatment should be undertaken early. Accurate, and, if possible, early diagnosis is therefore most essential. The following is a brief account of a typical example :—

A man, aged 43 years, had spent most of the last twenty years abroad, having been in India, Africa, Mauritius, Ceylon, and the West Indies. For the past eight years he had suffered from loose actions of the bowels, the motions being yellowish or pale, sometimes frothy, and sometimes large. The condition had been diagnosed as malarial in nature, or more frequently merely as 'colitis,' and lastly as 'sprue.' He had submitted to various methods and courses of treatment with little if any benefit, and was finally sent to me definitely as a case of intractable sprue. The stool was certainly somewhat suggestive of that disease, fatty, greasy, unformed and bubbly.

The blood, however, showed a normal total and a normal ionic calcium content, and the faeces nothing particular except fat in excess. A second stool was found to contain *Entamoeba histolytica* cysts, and the probabilities were that the whole trouble was a residual dysenteric condition, the fat being due to the milk diet to which he had been restricted for a long period. Injections of emetine and a course of emetine bismuthous iodide cleared things up. There was no sprue.

Cases like this are comparatively common and I have, therefore, for some months past been examining the blood from patients at the Tropical Diseases Hospital and elsewhere in order to see whether this peculiar condition of the calcium—reduction of the ionic with a normal or nearly normal total—occurred in other diseases in which there was diarrhoea as a prominent symptom especially diarrhoea of a sprue-like nature. The method employed has been that devised by Dr. H. W. C. Vines (1921).

The following Tables will save pages of description :

TABLE I

Showing the Calcium content of the serum of Sprue patients before treatment

Initials	Ionic Ca.	Total Ca.	Initials	Ionic Ca.	Total Ca.
	%	%		%	%
Fr.	7·7	10·1	Ly.	6·9	9·9
Td.	6·8	9·9	J.T.	7·9	10·0
C.	6·9	9·8	R.*	8·1	10·4
L.E.B. ...	6·3	10·1	B.	6·9	9·9
A.W.	7·3	10·4	Bp.	6·6	9·0
E.	7·3	9·9	Bl.	7·9	10·0
Ge.	7·1	10·8	Ln.	6·3	9·9
R.W.	6·3	9·9	Tr.	7·7	10·1
St.	6·6	9·8	Ws.	7·1	10·1
R.P.W. ...	6·9	9·8	Es.	7·9	9·9
McG.	6·1	9·9	H.S.	7·0	9·9
Cr.	7·9	10·1	McC.	6·6	10·0
Ln.	6·6	9·8	Ts.	7·7	10·5
Ct.	6·6	10·1	Hn.	6·6	10·2
Bn.	6·1	10·6	Ee.	6·4	10·4
Rl.	6·7	10·4	Cm.†	6·1	9·4

* A mild case ; had been under treatment outside

† Very ill ; died within twenty-four hours

TABLE II

Showing the Ionic Calcium of the serum of patients other than Sprue

Disease	Initials	Ionic Ca.	Disease	Initials	Ionic Ca.
		%			%
Dysentery Amoebic ...	Ke.	10·6	Dysentery Bacillary ...	P.L.W. ...	11·5
,, ...	Ma.	10·6	,, ...	Ka.	10·6
,, ...	Me.	10·4	,, ...	M.T. ...	10·6
,, ...	Cr.	10·0	,, ...	Wa.	9·9
,, ...	D.S.	10·4	,, ...	S.R. ...	10·4
,, ...	B.S.	10·6	,, ...	W.S. ...	10·4
,, ...	H.E.	10·4	,, ...	M.D. ...	10·6
,, ...	Sn.	9·9	,, ...	T.R.	10·6
,, ...	J.N.R.A. ...	11·0	,, ...	So.	10·6
,, ...	S.N.	9·5	,, ...	B.N.	10·4
,, ...	Co.	10·6	,, ...	Sm.	10·6
,, ...	C.N. ...	10·4	,, ...	Jn.	10·1
,, ...	W.N. ...	10·1	Mucous colitis	O'B	10·4
,, ...	P.T.	10·6	,, ...	N.N. ...	10·4
,, ...	J.N. ...	10·6	,, ...	B.	10·6
,, ...	K.F.	10·4	,, ...	G.R. ...	10·1
,, ...	Mi.	10·7	,, ...	F.N. ...	10·1
,, ...	T.R.	10·0	,, ...	Bi.	11·1
,, ...	Bk.	10·4	Ulcerative colitis ...	Br.	10·6
,, ...	Cr.	10·6	Syphilis ...	A.W. ...	10·6
,, ...	Jn.	10·0	,, ...	W.	10·6
,, ...	Pe.	10·4	,, ...	N.	10·6
,, ...	Ds.	11·1	,, ...	R.	10·1
,, ...	Pt.	10·4	,, ...	Wr.	10·4
,, ...	Sy.	10·2	,, ...	B.	10·6
,, ...	E.	10·4	,, ...	C.H. ...	10·6

TABLE II—*continued*

Disease	Initials	Ionic Ca.	Disease	Initials	Ionic Ca.
		%			%
Syphilis ...	Na.	10·4	Malaria (M.T.)	El.	9·8
,, ...	A.	10·6	,, ,, ...	Ko.	10·2
Malaria (B.T.)	C.	10·4	,, ,, ...	Bo.	10·4
,, ,, ...	C.E.... ...	10·6	,, ,, ...	To.	9·4
,, ,, ...	H.*	9·5	,, ,, ...	La.	10·6
,, ,, ...	D.	9·9	,, ,, ...	H.B.... ...	9·6
,, ,, ...	B.	9·1	,, ,, ...	Dn.	10·2
,, ,, ...	M.	10·1	,, ,, ...	Mn. ...	9·4
,, ,, ...	Eg.	10·4			
,, ,, ...	Bl.	10·4	Kala azar ...	Mo.	10·4
,, ,, ...	Om. ...	10·4			
,, ,, ...	Cr.	9·9	Trypanosomiasis	W.	10·1
,, ,, ...	Ch.	10·6	General Paralysis (treated by malaria)	Ma.	9·9
,, (M.T.)	Wa.* ...	9·1			
,, ,, ...	B.R.... ...	9·9			
,, ,, ...	W.	9·8	Filariasis ...	W.	10·1
,, ,, ...	B.O....	9·9	,, (?) ...	B.	10·6
,, ,, ...	S.	10·4			
,, ,, ...	C.	9·5	Beriberi ...	C.	9·9
,, ,, ...	A.	10·1	,, ...	F.C....	10·4
,, ,, ...	Pe.	9·2	,, ...	C.L....	10·4
,, ,, ...	Cl.	9·1	Jaundice and diarrhoea	D.	10·0
,, ,, ...	Ch.	8·9	,, ...	S.	10·2
,, ,, ...	Hd.	9·7	Undulant fever	B.	10·7
,, ,, ...	F.C....	9·7	Tuberculosis ...	P.	10·4
,, ,, ...	K.	9·7	Endocarditis ...	R.	10·4
,, ,, ...	R.	9·1	Tapeworm ...	S.	10·6
,, ,, ...	L.	10·1	Ankylostomiasis	L.	10·2
,, ,, ...	T.	9·1	Ascariasis, &c....	F.	10·6

* These had Syphilis also

B.T. = *Pl. vivax* infection.

M.T. = *Pl. falciparum* infection.

Table I is a list of cases of sprue whose blood was examined on their first coming to hospital or within a week or so, that is, before sufficient time had elapsed for any treatment to have had an appreciable effect on the calcium content.

From this it will be seen that the total calcium is but very little reduced, whereas the ionic calcium is between 20 and 30 per cent. below the normal.

Table II gives the amount of ionic calcium, as before in mgms. per 100 c.c. in the serum of patients other than sprue, many of them with diarrhoeic symptoms. Others have also been included as a matter of interest. It will be seen that those conditions most likely to be confounded with sprue, namely, the dysenteries and forms of colitis, are all about the normal limit as regards the ionic calcium. It is worthy of note also that the only common tropical affection in which there is a fairly consistent reduction is that of malaria, and in the one case of general paralysis of the insane which was being treated by malaria.* But in none of the malarial patients whose blood was examined was the reduction of the ionic calcium anything like so great as that found in sprue. This, moreover, is not of much importance in practice, for it would only be in those not very common cases in which diarrhoea was associated with malaria as a prominent symptom that the question of diagnosis would arise at all.

It is clear, therefore, that the estimation of the ionic calcium is a very useful factor in diagnosis of sprue from other diarrhoeal conditions, and, since accurate diagnosis forms the basis of rational treatment, a test which will establish the diagnosis of an obscure disease such as is sprue from others with sprue-like symptoms becomes of considerable medical importance.

Passing to the second part of this paper—the value of the estimation of the ionic calcium of the serum in gauging the progress of disease in Sprue.

Important as the test is in diagnosis, it is vastly more important and more useful as an indication of progress. If all is going well and the serum is examined at intervals of a fortnight, or, better still, a week, the percentage of ionic calcium is found to rise steadily to

* This is of interest seeing that cases of chronic malaria have been recorded of late in which marked improvement followed the administration of parathyroid.

the normal. Any return of symptoms, such, for example, as a sore or tender tongue, or the reappearance of a few aphthae in the mouth, is accompanied by a drop in the ionic, not the total, calcium. Such may arise from an attempt to increase diet too rapidly or unduly hurry the convalescence, and may be, usually is, a warning of a relapse, and, if regarded as a mere "upset from indigestion" and the warning allowed to pass unheeded, a relapse will certainly take place, necessitating the loss of several days, perhaps weeks, in the cure. If, however, the calcium content is determined and is found to have dropped, a return to milk for a couple of days or so will usually suffice to restore the balance and progress will then continue. If, on the other hand, it is found to have maintained its level, the symptom is of no practical importance and the fuller diet need not be curtailed.

To avoid repetition this aspect of the question may be dealt with briefly under the following headings :—

1. Ionic calcium is always low in the untreated disease, even in the early stages.
2. The ionic calcium increases as the condition improves.
3. The ionic calcium falls again if a relapse occurs.
4. Improvement takes place when calcium is administered alone, but is slower and less stable than when parathyroid is given in addition.
5. Improvement, evidenced by the clinical condition and, more accurately, by the rise in the ionic calcium, is more steady and more rapid when parathyroid is given.

1. This first point need not be further elaborated. It is abundantly proved by what has gone before in showing the value of the test in diagnosis, and the table (Table I) affords many examples.

2. The ionic calcium increases as the patient's condition improves. The following is an illustrative case.

J.T., aged 35 years, had suffered from sprue for eight months. His condition had been diagnosed, first as amoebic dysentery and, later, as colitis, but treatment for these had been unavailing. The symptoms were typical—sore mouth and tongue, with ulceration, flatulence, acidity, large, pale, frothy stools, cramps and loss of weight. At commencement of treatment the ionic calcium was low, 6·8 mgm. per cent.; three weeks later there was no longer any soreness of the mouth, the stools were less frothy and bulky, and the dyspepsia was less. The ionic calcium was now 7·9 per cent.; the total had remained about normal, 10·4 per cent. A fortnight later there was great general improvement; the patient felt

stronger, no longer suffered from lassitude or depression, had only one action of the bowels daily, and that was normal, was on a fairly generous diet and was getting up for three or four hours each day. The ionic calcium was now 8·8 per cent.; in yet another fortnight he 'felt well,' and the calcium was 9·5 per cent., and a week later 10·4, the same as the total calcium. He left the nursing home, taking full diet, and went to Scotland, where he played golf and, in fact, lived a normal life, and ceased to take any medicine. Two months later he wrote to say that he was keeping 'quite fit,' and four months afterwards he was in London and called to show himself. He looked the picture of health, and opportunity was taken to test his blood again. It had more than maintained the previous level, being now at the upper limit of the normal, namely 11·1 per cent. This patient has returned to the tropics.

3. The ionic calcium falls again below the former level if a relapse occurs.

F.W.W., 31 years; duration of typical sprue symptoms six months. When the blood was first examined, after three weeks' treatment, the ionic calcium was 8·1 and the total 10·6 per cent. He made excellent progress and the ionic calcium was found to be 10·6 per cent. three and a half weeks later. He was then allowed full diet and to do more or less as he liked, getting up and going about. In fact, he tried to go along too quickly. In two weeks the mouth began to feel sore, and the stools were a little more bulky and pale. He was, in short, starting to relapse. Another examination of his blood was made and it was found that though the total calcium had remained at 10·6, the ionic had fallen again to 8·5 per cent. The diet had to be reduced and treatment begun again. In another month he was well and able to go out, and there has been no report of any recurrence of symptoms.

This point is important enough to warrant the recording of a second case, which was more severe than the last.

A.C., female, 51 years, had suffered for two years or more from sprue, with frequent relapses. All the typical symptoms were present, the mouth symptoms—soreness, tenderness and ulceration of tongue and buccal mucous membrane—being very pronounced. The ionic calcium before she started treatment was as low as 6·9 mgm. per cent.; three weeks later all the symptoms were much improved. She felt stronger and was getting up for an hour or two daily, and the ionic calcium had increased to 8·1 per cent. In another fortnight it had reached normal, 10·6, and a fortnight later still had increased to what is regarded as the upper limit of the normal, 11·6 per cent. Four weeks later the tongue began to feel tender, two small aphthae appeared, and she began to pass paler motions. The blood was sent up and it was found that the ionic calcium had fallen again to the lower limit of the normal, 10·2 per cent. A return to milk for three days, with a resumption of the medicine, cleared up these symptoms, which, if disregarded, would, as on former occasions, most certainly have been the forerunners of a severe relapse.

4. The mode of treatment has a distinct effect upon the rate of progress, and this is best gauged by the ionic calcium estimation. The disease having been shown to be associated with a deficiency in this substance, treatment by calcium in some form leads to improvement of symptoms, though, if given alone (that is, without

parathyroid) this improvement is slow and not very stable. As an example of the former the following may be very briefly narrated :—

G., male ; a fairly severe case treated on the ordinary lines—diet (chiefly milk) and rest in bed, but without parathyroid. After ten weeks, the ionic calcium was only 7·1 per cent., though improvement had been steady but very gradual. Three weeks later it had risen to 8·3 per cent. only, and in another three weeks (i.e., after sixteen weeks' treatment) it was still below normal, namely 9·1 per cent., though the total was 10·6. Clinically speaking, he was nearly well, was up and about, but on a limited diet still, and shortly afterwards he left hospital. Unless he is very careful he is almost certain to relapse, and that probably before very long.

The following is an illustrative example of the instability of treatment by calcium alone.

H.B., 40 years. After twelve weeks in hospital this patient was up and about, though on a restricted diet, and was to all appearances well, and was on the point of leaving. Without any reason being discovered, and while he was on the restricted diet and under a cautious régime, he again lost weight and expressed himself as 'not feeling quite so well.' His blood was taken and the ionic calcium was found to have fallen to 9·5 per cent., though the total was normal, 10·6 per cent. The diet was therefore again reduced, and he was made to return to bed ; in three weeks the ionic calcium was again normal, 10·4, but he was not considered well enough to leave hospital for another two months.

These two cases are good instances for showing that it is not absorption of calcium which is defective, but the proper regulation of it after it has been absorbed.

5. Lastly, the improvement, as evidenced both by the clinical condition and by the rise in the ionic calcium of the serum, is more rapid when, in addition to the administration of the calcium, parathyroid is also given to regulate its metabolism. Previous papers (see References) afford many examples of this, but to render the present paper more complete the following may be briefly narrated.

(1) A. McG., male, 28 years of age. Ill for ten months with the typical symptoms of sprue. He had lost 28 lbs., in weight, had a sore and tender tongue and mouth, and was passing frothy, pale and rather bulky stools. When admitted to hospital his ionic calcium was down to 6·1 mgm. per cent. He was given calcium in the form of milk and also cachets of the lactate, gr. 15, thrice daily, and parathyroid, gr. 1/10 of the dried extract, twice a day. He reacted wonderfully well. In a week the ionic calcium was 8·1, and in a fortnight 10·1 per cent., the total being close upon normal, 9·9 per cent. on the first two occasions, and 10·1 on the third. He remained in hospital for another five and a half weeks, steadily maintaining his improvement, and left after a stay of less than eight weeks. The ionic calcium when he went out was 10·8 per cent.

(2) S., male, 48 years. This was a more severe case and one of longer standing, having existed for over two and a half years. When admitted to hospital he was 36 lbs. below his normal weight. He was at once given the same treatment as the patient whose case has just been described—calcium lactate gr. 15, three times, and extract parathyroid gr. 1/10, twice daily. The symptoms steadily and rapidly improved and he left hospital thirty-one days after admission, feeling and looking very well.

After one week's treatment the ionic calcium was 7·0 per cent.; after two weeks 7·4; after three weeks 10·8, and it had remained there when he left. More than six months later he came to show himself. He had taken no medicine during that period and had kept perfectly well, and had not had to restrict his diet or smoking in any way. He was a heavy smoker and the soreness of the mouth, by depriving him of this solace, caused him great distress. His blood was taken to see if the calcium content had been maintained, and it was found that his own parathyroids were carrying on their work satisfactorily, and that absorption of calcium was very good. The ionic and the total were the same, 11·1 per cent.

(3) A.B.W., female, 34 years. This case, though not of so long duration, only twelve months, was exceptionally severe. At times she had as many as fifteen copious, loose, frothy, pale stools in the twenty-four hours; her tongue was very sore and ulcerated; she had troublesome cramps, and had lost 42 lbs. in two months. She then started calcium lactate and parathyroid, but both of them in too small doses; of the former gr. 5 thrice daily, of the latter gr. 1/20 twice. When seen two months later, on her arrival from abroad, the tongue and mouth were very sore, red, and tender, and she was obviously ill. The stools, however, had been reduced to four daily. The ionic calcium was at this time 7·3 per cent. She was at once put on an increased dose of calcium, namely gr. 15 of the lactate three times a day, and the dose of the parathyroid was doubled. The change was remarkable; within fourteen days the number of stools was reduced to one daily, no longer frothy, and much less bulky. In another week she had to take liquid paraffin to overcome constipation, and was progressing so well that she was able to sit up for some hours each day. The serum calcium (ionic) had increased to 9·5 per cent.; in another fortnight she was going for walks, was taking a diet comprising milk and milk puddings, bread and butter, eggs, fish, chicken and fruit. The stools were normal in size and colour, and only one in the twenty-four hours. The ionic calcium was now 10·1 per cent. Two weeks later, that is seven weeks after I first saw her, she came to London from Bristol, looking well, and taking all food without discomfort. Another blood examination showed a normal calcium content, 10·6 per cent. It was considered that the food now contained abundant calcium, and, since absorption had not been upset, the total having been practically normal throughout, this element was omitted from the medicine. The dose of parathyroid was reduced in order to test whether her own glands were now capable of carrying on their function and instructions were given that, if no untoward symptoms arose, it, in turn, was to be stopped altogether after another week. This programme was carried out and three weeks later she again travelled up from Bristol, this time to visit the Wembley Exhibition. She had had no treatment of any kind during the previous fortnight, but the calcium content of the blood was fully maintained, being now 11·1 per cent.

I saw her finally four weeks afterwards; she looked the picture of health, stated that she felt better than she had done for years and full of energy, that she was going about all day, eating anything put before her; in fact, living a normal life, and was arranging to return to India.

A last examination of the blood was made and gave ionic calcium 11·1 per cent., no residual, coagulative, combined calcium. This patient wrote five months later to say that she was in perfect health, eating heartily without any restrictions, had more than maintained her weight, was doing hard work (as a missionary) and 'felt full of energy.'

These facts are demonstrated in the accompanying Tables III and IV.

TABLE III

Sprue Cases under Ordinary Treatment
Showing the Gradual Rise in the Ionic Calcium

Initials	Ionic Calcium in mgms. per 100 c.c. serum.												
	Before treatment	Number of weeks after starting treatment											
		1	2	3	4	6	8	10	12	14	16	18	20
F. ...	...	7·7	...	...	9·1	...	...	...	...	...	...	...	...
L. ...	...	...	...	6·9	...	...	9·1	...	...	10·1	...	...	...
Ln. ...	6·6	...	...	...	...	9·9	...	...	...	...	...	...	...
H.W.B.	...	...	...	...	...	...	...	...	9·3	...	10·4	...	...
G. ...	...	...	...	...	...	...	7·1	...	...	8·3	...	...	9·1
L.O. ...	...	6·3	...	...	...	...	...	9·9	...	...	...	...	...

TABLE IV

Sprue Cases Treated by Parathyroid in Addition
Showing the more rapid Return of the Ionic Calcium to Normal

Initials	Ionic Calcium in mgms. per 100 c.c. serum								
	Before treatment	Number of weeks after starting treatment							
		1	2	3	4	6	8	10	12	Remarks
J.T. ...	...	7·9	...	8·8	...	9·5	10·4	...	11·1	Still 11·1 when seen 4 months after ceasing to take any medicine.
M.C....	6·9	...	8·1	...	10·5	...	11·6	...	...	
A.W....	...	7·3	...	9·5	...	10·1	10·6	...	11·1	Maintained when seen 2 months after. Reported as 'perfectly well' 7 months after.
H.E. ...	...	7·3	...	...	...	...	...	10·6	...	
E.J.W.	...	...	8·0	...	8·9	10·4	...	10·8	...	11·0 when seen 3 months after ceasing medicine.
McG.	6·1	8·1	10·1	...	...	10·8	...	...	...	
H.S. ...	...	7·0	7·4	10·8	10·8	...	...	...	...	11·1 when seen 6½ months later.
W.E. ...	6·9	7·4	8·7	9·7	10·4	...	...	...	...	Living a busy, active life and 'feeling full of energy' 6 months later.
S. ...	6·6	...	7·7	...	9·7	10·4	...	...	...	Gone abroad; keeping well.
E. ...	...	...	7·1	...	10·3	...	11·1	...	...	

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SUMMARY

1. Sprue is a disease which is constantly associated with a fall in the amount of ionic calcium in the serum, whereas the total remains at or about normal.

2. A rise and fall of the ionic calcium coincides with improvement and relapse.

3. Absorption of calcium is little, if at all, interfered with, but calcium metabolism is upset.

4. Consideration of this fact and of some of the other symptoms of sprue, especially the cramps and tetany in severe or advanced cases, points to interference with the function of the parathyroid glands.

5. Amelioration of symptoms, followed by cure, is obtained by oral administration of suitable salts of calcium and a pure and active preparation of parathyroid in adequate doses.

6. The period needed for cure by these means is much shorter than by previous methods, the main symptoms clearing up in some cases within a few days. The administration of the parathyroid must, however, be maintained to stabilise the amelioration, but no ill-effects have been found to occur if it be continued longer than is actually necessary to bring about this result.

7. In the light of the above it is interesting to note that most of the old, empiric remedies contain lime as an important constituent.

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MALARIA PARASITES IN THE PLACENTAL BLOOD

BY

B. BLACKLOCK

AND

R. M. GORDON

(From the Sir Alfred Lewis Jones Research Laboratory, Freetown, Sierra Leone)

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During the months of July, August and September, 1924, the examination of twenty-six placentas for malaria parasites was carried out among native women in Freetown.

EXAMINATION OF THE PLACENTA

The placenta arrived at the Laboratory at variable periods after delivery, sometimes within an hour and almost always within twelve hours. An incision was made through a cauterized area on the placenta, blood was withdrawn from the bottom of the cut by means of a pipette, films were spread, and stained either with Leishman's or Giemsa's stain. Malaria parasites were found in twelve, i.e., 46 per cent of the twenty-six cases.

EXAMINATION OF MATERNAL PERIPHERAL BLOOD

The maternal peripheral blood was examined at the time of the birth in twenty-three of these cases, with the result that only four, i.e., 17 per cent. were found positive. No case of infection of the maternal peripheral blood was found in which the placental blood failed to show a much heavier infection.

Among the twenty-six cases there were four accidents (premature birth, etc.). In the twenty-two cases which had normal labour there were nine, i.e., 41 per cent., in which the placental blood showed parasites, whereas in the four cases which had abnormal labours there were three, i.e., 75 per cent., in which the placenta showed parasites.

EXAMINATION OF THE UMBILICAL CORD

The umbilical cord was cut and films made from the blood in the vessels at two places, one as near as possible to the placenta and one about six inches from the placenta. In none of the twenty-six cases was infection of the umbilical cord blood found, in spite of the fact that in all cases where the placenta was heavily infected several thick films were examined, in addition to thin films; nor was infection found in the veins of the membranes.

EXAMINATION OF CHILD

Films of the peripheral blood of the new-born child were made in twenty-four of the twenty-six cases; no infection was found.

In order to obtain information with regard to the possibility of congenital infection in the new-born child having been overlooked in the examination of cord and peripheral blood films, additional examinations were made.

1. In two cases of death of the child where the placenta of the mother was infected, an exhaustive search was made of smears of the heart blood, thymus, lungs, liver, spleen, bone marrow, and omentum with negative result.

   In two similar cases where liver puncture alone was permitted, the smears proved negative.
2. Repeated examination of children (born of mothers with placenta infected) was carried out up to a week after birth and proved negative; thick as well as thin films being examined.
3. The blood of forty-one children aged one month or under was examined; infection was found in only one case, a child between three and four weeks old. Such a case as this cannot, for reasons given below, be classified as congenital.

Pezopoulos and Cardamatis (1907) drew attention to the heavy infection which may occur in maternal blood in the placenta at a time when the peripheral blood has only a few parasites. The preponderance of schizogony forms in the placental blood was noted by these observers. Their examinations of the umbilical cord blood, the peripheral blood of the child and organ smears led

them to conclude that the malaria parasite does not pass through the placenta from the maternal into the foetal circulation.

Clark (1915) examined films of the placental blood in a series of 400 cases, taking the blood from the maternal aspect of the placenta after the removal of clots. By this method he found *P. falciparum* in nineteen, i.e., 4·7 per cent. of the cases. Compared with this his findings of parasites in the maternal peripheral blood of the same cases taken at the end of labour were positive in only eight, i.e., 2 per cent. of the cases. In all the cases where the peripheral blood was positive, the placental blood showed a much heavier infection. Among the 400 cases there were forty-four accidents (abortion, still-birth, premature labour). In the 356 cases which had normal labour there were twelve, i.e., 3·4 per cent. in which the placental blood contained parasites, whereas in the forty-four cases which had abnormal labour there were seven cases, i.e., 16 per cent. in which the placental blood contained parasites.

In addition to the placental and maternal peripheral blood, Clark examined the blood in the umbilical cord. The cord was carefully cleaned, cut across and films were made from the foetal blood. In only one case were parasites found in blood from the cord ; in this case the maternal peripheral and the placental bloods were both heavily infected, and the author considers it was due to the complication of an associated accident of pregnancy that the child had congenital infection.

CONGENITAL MALARIA

It is generally acknowledged that congenital malaria is a very rare condition. By congenital malaria is meant malaria which the unborn child acquires from the mother owing to failure of the barrier action of the placenta. The mechanism of the failure on the part of the placental barrier, i.e., whether it is due to the ability of exceptional parasites to penetrate through a healthy placenta, or to disease of, or accident to the placenta during pregnancy, does not here concern us.

The important point to decide is whether, in any given case, there is sufficient evidence to prove that it can only have been acquired owing to failure of this barrier action. Cases in which

parasites are found in the vessels of the umbilical cord at birth or in the peripheral blood or organs of the child at birth clearly belong to this category.

The more remote the period after birth at which parasites are found in the child the less justification have we for speaking of congenital malaria.

There are at least two definite and distinct ways in which a child not infected congenitally may become infected. The first way is by inoculation through abrasions of the skin in the process of delivery, where the maternal blood is mechanically inoculated into the child; the second way is by the bites of infected mosquitos. Before any case of malaria can be established as congenital, it is clearly essential that the possibility of infection by either of these means should be absolutely excluded. This will be in many cases a difficult task, but the onus of proof is on those who claim cases as congenital. There are many cases standing in the literature to-day as cases of congenital malaria which are represented as congenital on altogether insufficient evidence.

It does not appear permissible to argue that the new-born child of a malarious mother is partially and temporarily tolerant and thus to explain the late development of symptoms and the late discovery of parasites in the child. There is little, if any, evidence to support this belief at present; on the contrary, there does exist a small amount of definite evidence which tends to disprove it, namely, those cases in which parasites are present in the blood of the umbilical cord at birth, in the peripheral blood of the child at birth, and also some cases in which the parasites were found at the first examination of the child's blood a few days after birth. Such cases are all against the theory of partial immunity of the new-born child.

What is to be our criterion in judging whether a case is or is not congenital? Our only available criterion is the minimum incubation period for the parasite, whether after inoculation of blood or after the bite of infected anophelines.

For example, in Yorke and Macfie's series of experimental infections with *Plasmodium vivax* by blood inoculation and mosquito bite infection, the shortest parasitic incubation found was after inoculation of infected blood, and was six days. We do not know whether, in the case of new-born children, this incubation period

might not be less. But accepting, for the present, six days as a minimum incubation period for *P. vivax*, it is not legitimate to assume to be congenital any case of infection with *P. vivax* in which parasites are first found more than six days after birth, unless other proof is supplied sufficient to show that the placental barrier has broken down.

When we consider the massive sporulating infections which the placental blood frequently shows (see Table I) and the absence of parasites in the cord and in the peripheral blood of the child, it appears certain that the walls of the villi are very efficient safeguards against the passage of merozoites even when these are present in enormous numbers. From the cases studied here, as well as from the literature dealing with congenital malaria, it appears that this condition is of great rarity.

THE PLACENTA AS AN INTERNAL ORGAN

In relapsing malaria, the parasites, in the intervals during which they cannot be discovered in the peripheral blood, are considered by most observers to be present in the internal organs. Of these, the spleen has always been the organ chiefly incriminated, the liver and bone marrow to a less extent have also been considered reservoirs. In the case of the spleen, however, it is known that even after removal of the organ, malaria may occur without re-infection. Obviously, in these cases, the spleen is not the only if even one of the chief reservoirs of the parasite. Acton, Knowles and Gupta (1923) punctured the spleen in fifteen cases and found splenic puncture to be ' a method of no diagnostic value in chronic malaria.'

In the present series an examination of the blood of the placenta has proved of striking value as a diagnostic of malaria. We recall that whereas examination of the peripheral blood revealed only 17 per cent. positive in twenty-three cases examined, the examination of the placenta revealed 46 per cent. positive in twenty-six cases.

NUMBERS AND STAGES OF PARASITE FOUND IN THE PLACENTA

In some of the blood films taken from infected placentas the number of parasites present is, as previously noted, quite remarkable. It might be approached rarely by the peripheral blood in fulminating cases of malaria, or more frequently by the capillary blood in

cerebral cases. We have never seen anything comparable to it in smears of spleen, liver, lung, kidney or bone marrow. Examination of the placenta at the time of child-birth, provided means of obtaining in women a malarial infection rate far higher than that obtained by even frequently repeated examinations of the peripheral blood.

The forms of parasites found in the placenta represent many stages not normally found in the peripheral blood. In all of the twelve placentas, parasites in the sporulating stage were present. In six cases sporulating forms were predominant, and in some of these, such forms constituted about 90 per cent. of all parasites found. In four of the other six cases there were about equal numbers of sporulating forms and young and medium-sized trophozoites. In two, the form of parasite present was almost exclusively the young trophozoite.

Double infection of cells was frequent, and in some instances two parasites in the same cell were sporulating at the same time.

COMPARISON OF INFECTION IN PERIPHERAL AND PLACENTAL BLOOD

The relative number of infected red blood corpuscles in the maternal peripheral and placental blood were determined in each of the four cases in which both the peripheral and placental blood contained parasites Ten thousand red corpuscles in the peripheral blood were counted and five hundred in the placental. The results are shown in Table I.

TABLE I.

Showing the ratio of the numbers of parasites in the peripheral to those in the placental blood, and the percentage of infected cells in each, and the ratio thereof.

	Peripheral blood	Placental blood	Percentage of red cells infected		Ratio
			Peripheral	Placental	
Case I	1	1292	0.05	65.0	1 : 1300
Case II	1	44	0.05	2.5	1 : 50
Case III	1	20	0.03	0.6	1 : 20
Case IV	1	1395	0.04	56.0	1 : 1400

CRESCENTS

It was notable that in none of the placenta infections were crescents seen, nor were they found in the peripheral blood where infected. When it is borne in mind that enormous numbers of parasites were examined in the placental blood in both thin and thick films, it seems reasonable to conclude that however favourable the conditions present in the placental blood may be for the development of asexual forms, they are, for some reason, unfavourable to the development of mature sexual forms.

That this absence of crescents was not a seasonal phenomenon affecting equally all cases is shown by the fact that, during the same period, crescents were present in 17 per cent. of ninety-six specimens of the peripheral blood examined in the children's clinic. The absence of crescents in the series of placentas examined here may be compared with the rarity of crescents recorded in the series of placentas examined by Clark.

ENUMERATION OF MEROZOITES PRODUCED BY PARASITES IN PLACENTAL BLOOD

Forms in which the process of sporulation was judged to be complete were chosen for counting; viz., those in which each merozoite was definitely separated from its neighbours. The maximum, minimum and average number of merozoites produced by the parasites of each case was thus determined. The highest count obtained was thirty-eight, but this was excluded owing to the possibility of its having a double infection of the red cell. The results are set out in Table II.

TABLE II.

Numbers of merozoites produced by parasites in different cases.

	Total number of sporulating forms counted	Maximum number of merozoites produced	Minimum number of merozoites produced	Averages
Case I	36	20	10	15
Case II	36	26	15	19
Case III	36	30	21	25
Case IV	36	33	20	26

It will be observed that Cases III and IV, as contrasted with Cases I and II, show a far higher average number of merozoites per parasite when division is apparently completed, and also that the maximum and minimum figures obtained in each of these two cases are at a higher level. Attempts were made to ascertain by measurement the size of the merozoites, but this proved unsatisfactory. Even where the largest numbers were produced, e.g., thirty-three merozoites, there was much variation in the size of individuals, and such merozoites appeared on the average to be equal in size to those occurring in parasites producing a much smaller number. It appears possible that certain varieties of *P. falciparum* produce a larger number of merozoites than others ; if this were so, it might have some bearing on the rapidity of the onset and the course of an attack.

Very little evidence is procurable as to the bio-chemical conditions prevailing in the placental blood ; while it is generally admitted that the blood of the placenta differs in some respects both from the maternal peripheral and foetal bloods, the differences do not appear to have been accurately determined.

Regarded purely from the standpoint of the suitability for the development of *P. falciparum*, the placental blood appears to afford conditions which are not paralleled in the maternal peripheral blood. These conditions appear extremely favourable for the asexual phase of development, but not favourable for the sexual phase. The placental blood fulfills the following conditions known to be necessary for the culture *in vitro* of *P. falciparum*.

1. Stagnation of blood.
2. Limitation of oxygen.
3. Presence of glucose.

Yoshida and Ko (1920) have shown that in all types of malaria the blood sugar is increased during the pyrexial period ; the maximum figure obtained was in infection with *P. falciparum*. Wells (1920) says glycogen is most abundant in the uterus at the time of childbirth and is abundant in the placenta.

It is interesting to recall the observation of Bass and Johns (1913) that the blood sugar of diabetics who have malaria renders the addition of dextrose to the culture medium unnecessary. It is perhaps relevant to remark that crescents have never been seen *in vitro* in media fulfilling the above-mentioned conditions.

SUMMARY

1. Of twenty-six placentas of native women examined in Sierra Leone twelve, i.e., 46 per cent., were infected with *P. falciparum*.

2. The infection in many of these placentas was massive.

3. Examination of the peripheral blood of twenty-three of these cases revealed only four, i.e., 17 per cent., infected.

4. In the placental blood, sporulating parasites were numerous and also young and half-grown forms.

5. Definite differences were observed in the number of merozoites produced by the parasites of different cases; these differences may influence the rapidity of onset and the course of the disease.

6. Crescents were never found in the placental blood in any of the cases examined.

7. No case of congenital malaria was encountered.

8. Some evidence is produced which suggests that malaria infection of the mother predisposes to accidents during pregnancy or at birth.

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A NOTE ON TWO VESICANT BEETLES BELONGING TO THE FAMILY *STAPHYLINIDAE*

BY

R. M. GORDON

(*From the Sir Alfred Lewis Jones Research Laboratory, Freetown, Sierra Leone*)

(*Received for publication 22 December*, 1924)

PLATE I

The vesicant properties possessed by beetles belonging to the family CANTHARIDAE are well recognised in medical literature, but references to the lesions produced by members of the family STAPHYLINIDAE are less common ; it, therefore, appears of interest to record the relatively severe ulcerations which have been observed to follow contact with two members of the latter family.

PAEDERUS AMAZONICUS (Sharp)

This beetle was first encountered at Manáos, Amazonas, in January, 1921, and appeared to be common in this locality at all seasons of the year. It was most frequently seen close to the river banks and sometimes proved a positive pest on the river steamers. It is a small insect only eight to ten millimetres long, the head, wing-cases and last two segments of the abdomen are of a blue-black colour, the thorax, legs and remaining segments of the abdomen being of a bright orange. The insect was well known to the natives under the name of ' Poto ' (pronounced Potā) ; they stated that if it alighted on the bare skin it produced a blister which sometimes developed into a slowly healing ulcer, the most common site of the attack being the face and neck ; they believed that it sometimes produced permanent blindness in young children when it found its way into the eye.

The writer was not able to verify any such injury, but as *Paederus amazonicus* is a small, free-flying and intensely active insect it seems quite probable that it may sometimes alight on the conjunctiva and there produce similar lesions to those later described in the text as probably caused by *P. sabaeus*. Göldi (1913) draws attention to a similar species *Paederus goeldii* (Wasmann, 1905) taken by him on the Rio Purus, also known locally as 'Poto' and possessed of similar vesicant properties, while da Silva (1912) describes another member of the same genus *P. columbinus* as causing dermatitis in Bahia. The only reference to *P. amazonicus* that the writer has noted is that of Bequaert (1921) who mentions it amongst a list of vesicant STAPHYLINIDAE compiled from various parts of the world.

The irritant powers of *P. amazonicus* were frequently tested on two Europeans and the results may be summarised as follows. When the insect was allowed to wander freely over the bare arm it produced no reaction, either immediate or delayed; if, however, it was irritated, or rubbed against the skin, after an incubation period of eighteen to twenty-four hours, a series of bullae made their appearance; these usually coalesced to form a single blister which burst, leaving an intensely raw and tender area which did not heal for about ten to fourteen days; the most severe reaction always followed vigorous rubbing of the beetle against the skin. When one of these insects was gently compressed in a live-box and examined under a dissecting microscope, minute drops of fluid could be seen exuding from the labial orifice and drying with extreme rapidity on the glass; no fluid was observed to be extruded from the anus or leg joints.

PAEDERUS SABAEUS

During May and June, 1924, Dr. E. J. Wright, of Freetown, called the attention of the Laboratory to several cases of ulceration of the face and neck which he thought might be caused by some vesicant insect; during June of the same year the writer observed a beetle which appeared to resemble closely the Amazonian species already referred to and which has subsequently been identified as *Paederus sabaeus*.

Rodhain and Houssiau (1915) give a description of the lesions produced by a vesicant beetle of the genus *Paederus* in Léopoldville. Bequaert (1921) states that this insect is *P. sabaeus*. Ross (1916) records similar reactions from Nairobi, due in this instance to *P. cribipunctata*, and Eysell (1913) records the vesicant properties of *P. peregrinus* in Malaysia.

P. sabaeus—unlike the Amazonian species—appeared to be quite unfamiliar to the local inhabitants and none of them associated its handling with any subsequent ill effects; Professor Blacklock took some specimens with him during a tour of the Protectorate and showed them to many of the natives, all of whom he informs me failed to recognise it. The insect appeared to be fairly common in Freetown during June, July and August; it disappeared during September and October, but re-appeared in the middle of November. Most of the specimens were taken at night-time in the Laboratory where they appeared to be attracted to the artificial light; usually as many as half-a-dozen beetles could be captured in the course of a single evening, whereas during the four months they were present in Freetown only three were taken in daylight. The periodicity of the insect is decidedly curious; thus, though careful search was made, not a single specimen was captured during September, October, or the first half of November, yet on November 21st no less than twenty-four specimens were taken in a space of two hours round a single electric light; the same locality the following night yielded only two of these beetles. The experimental results obtained with this beetle were very similar to those recorded with *P. amazonicus*, but of a slightly milder nature; also the incubation period appeared to be longer, no trace of any reaction occurring for a full twenty-four hours and blisters not appearing till after the lapse of two days, the subsequent course of these blisters being similar to that already recorded for *P. amazonicus*: they leave a well-marked cicatrix, some of the scars being still clearly visible five months after the experiment. It has already been noted in the case of *P. amazonicus* that on compressing the insect, fluid (apparently of a volatile nature) could be observed exuding from the labial orifice; on testing *P. sabaeus* in a like manner no such result was noted. By means of a razor one of the beetles was divided into three separate portions consisting of the head, thorax and abdomen; each portion

was then rubbed into a different part of the forearm ; a well-marked reaction subsequently developed on the areas smeared with the thorax and abdomen, but none where the head had been applied. Göldi (1913) refers to an enteritis occurring in the Marshall Islands under the name ' Toddy-Krankheit,' which is supposed to be due to the swallowing of fluids into which some vesicant beetle has previously fallen ; in order to test the toxic nature of *P. sabaeus* in this respect one of the beetles was ground up in two c.cs. of tap water and the fluid injected down the oesophagus of an adult guinea-pig ; no results followed the injection, the animal remaining well and the stools formed.

Dr. Wright has recently brought to my notice an interesting case in which the lesions would appear to be due either to this insect, or else to some similar species possessed of equally strong vesicant powers. Mr. G. was motoring in Freetown and about seven in the evening was struck in the eye by some small object which he took to be an insect ; he rubbed it out of his eye and only suffered temporary inconvenience. The next day the eye was slightly inflamed and sore, the following day it was considerably worse and he consulted Dr. Wright, who was at once struck with its similarity to the cases he had previously observed on the face and neck ; at this time the eye was intensely inflamed and discharging freely ; a circle of inflammation and oedema extended all round the eye and involved the eyebrow and the cheek. On examining this latter area with a lens, numerous minute bullae could be seen precisely as in the case of the experimental lesions already referred to. The following morning—i.e., sixty hours after the injury—the blisters were greatly increased in size and a further crop had made their appearance on the left ear, which was swollen and tender ; these latter blisters were in just such a position as would be caused by a person brushing some inflammatory substance across the face from the eye to the ear.

I am indebted to Mr. K. G. Blair, of the British Natural History Museum, for the identification of both these insects.

Since the above was written the writer's attention has been drawn to an article by Strickland (1924) which gives an account of the vesicant properties of *P. fuscipes*, as studied in India. In both morphology and habits—as regards attraction to light, etc.—this insect appears to resemble closely *P. sabaeus*. The dermatitis and preceding incubation period being also similar.

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EXPLANATION OF PLATE I

Fig. 1. Showing dermatitis produced by *P. amazonicus*. Photo. taken twenty-four hours after infection.

Fig. 2. Showing dermatitis produced by *P. sabaeus*. Photo. taken three days after infection. The lesions shown at 1 and 3 were produced respectively by rubbing in the thorax and abdomen; no reaction followed the rubbing in of the head at 2.

Fig. 3. Showing dermatitis involving the eye and ear, probably caused by *P. sabaeus*. Photos. taken two and a half days after infection.

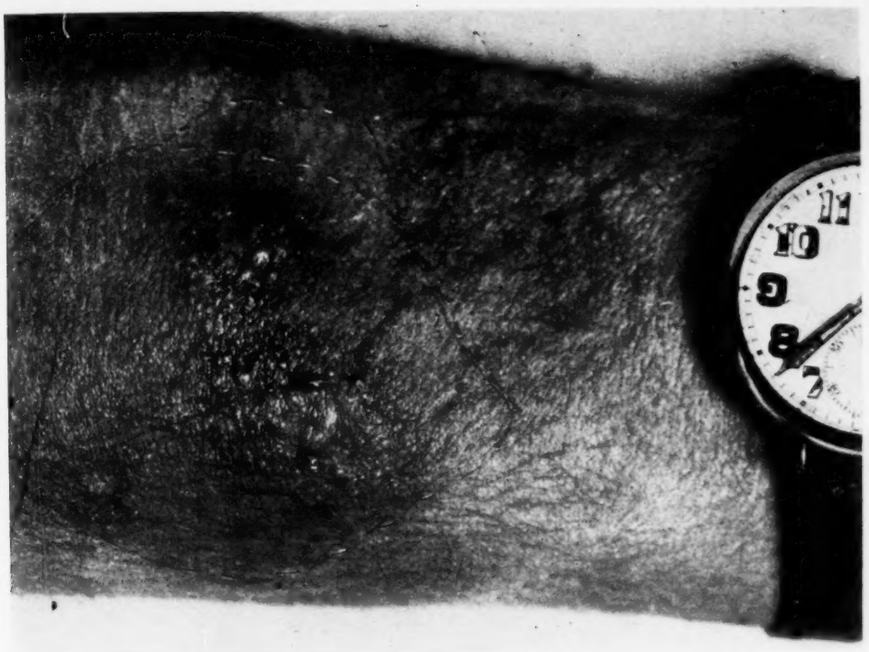

FIG. 1

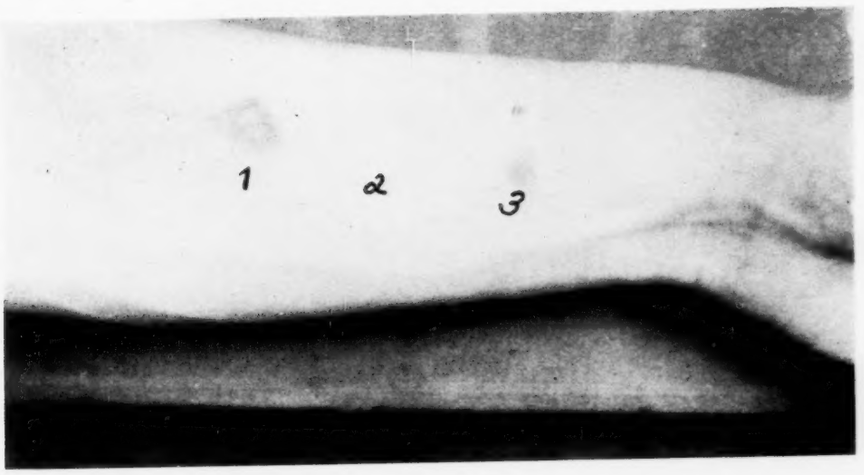

FIG. 2

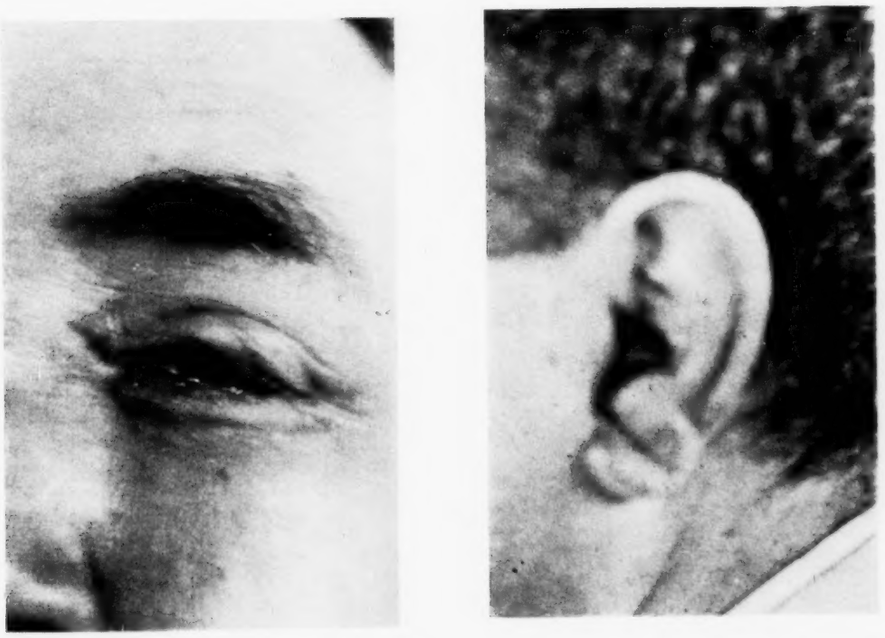

FIG 3

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THE GENUS *KILULUMA*

BY

E. L. TAYLOR

(*Received for publication* 24 *December*, 1924)

This genus was the subject of a recent paper by Thapar (1924), who divided it into six new species: unfortunately, he does not give any key to assist in placing a member of the genus in its proper species, nor does he give any list of differences of specific value, but only a detailed description of each type, in which he singles out but very few points considered by him to be of specific importance. A complete list of the measurements and morphological differences given by Thapar was, therefore, drawn up with the idea of preparing a key to be used in the classification of worms of this genus in the Museum of the Liverpool School of Tropical Medicine. On perusal of this table of differences there appeared to be small reason for the subdivision of the genus to such an extent, and the subsequent examination of the large amount of material at my disposal has brought me to the conclusion that the individual differences noted by Thapar are only sufficient to divide the genus into two, or possibly three species. The Museum of the Liverpool School of Tropical Medicine contains some eight hundred worms of the genus *Kiluluma*, collected in Rhodesia from five rhinoceroses; measurements were made from a number of worms picked at random; details of morphology noted in a still greater number, while general characters of the whole collection were also noted for the purposes of this paper.

Of the six species named by Thapar, I should consider the following four synonymous:—*K. rhinocerotis*, *K. africana*, *K. pachyderma* and *K. solitaria*, since in the same individual I have found varying combinations of the supposed specific differences. These supposed differences in morphological characters are very small. As an example, two definite points, in which the presence or absence of a character is involved, may be singled out, namely

the presence or absence of a second wing to the spicules, and of a small branch to the externo-dorsal ray. Although, according to Thapar, the presence of the branch to the ray should only coincide with a one-winged spicule (*K. pachyderma*), I have frequently found it to coincide in the same individual with a two-winged spicule.

Differences between these four species in the matter of the detailed measurements given by Thapar are also very small, and similar measurements made from material at hand have in no single instance fitted one of the four species to any marked degree more than the rest ; where measurements of one part of an individual might coincide with those of *K. rhinocerotis*, measurements of other parts might fit *K. pachyderma*, or *K. solitaria*, or *K. africana*. In my opinion, Thapar attaches too much importance to small differences in measurement : for example, in the text, attention is especially drawn to the larger spicule in *K. africana* as a difference from *K. rhinocerotis*, yet this difference is only between spicules 2·1 mm. and those 2·25 mm. in length, where the male of the first species measures 13 mm. and of the second 13 to 14 mm. in length.

Differences made on the position of the so-called ' filiform process of the lips ' and the narrow, or the swollen appearance of the anterior end of the ' lips ' do not seem to hold, since this internal leaf-crown appears to be pliable and liable to be fixed in varying positions. Although by far the greater number of worms examined by me showed the ' lips ' in the position seen in Thapar's drawings of *K. pachyderma* and *K. macdonaldi*, I came across several with ' lips ' approaching the shapes shown in the drawings of *K. africana* and *K. rhinocerotis*. I did not, however, come across any with ' lips ' in the positions seen in the drawing of *K. solitaria*.

The reasons for making the species *K. macdonaldi* do not seem to be much stronger than those for making the four other species mentioned above ; but two characters are described as not occurring in these four ; firstly, the cervical papillae are said to be anterior to the excretory pore ; and secondly, the preventral ray in the bursa of the male is stated to be moved forward to the position of a prebursal papilla. The first of these two differences does not seem to be of great importance, since in common with other species the papillae are at about the same level as the excretory pore. The second point may be of more importance, although I have come

across some remarkable variations from the normal in the arrangement of bursal rays ; two males actually showed asymmetrical lateral lobes, the postero-lateral and extra-lateral rays being present on the one side only.

The sixth species, *K. magna*, shows some outstanding differences, the most marked of which is in the much greater length of the oesophagus, the excretory pore and cervical papillae being on that account in the oesophageal region of the body : the general size of the worm is greater than in the five preceding species, the uterus is much larger and the eggs are double the size. I did not find any worm belonging to this species, but the differences given by Thapar clearly set it apart from the other five.

In my opinion, *K. rhinocerotis*, *K. africana*, *K. pachyderma* and *K. solitaria* are one and the same species to which *K. macdonaldi* may also belong, while *K. magna* only has distinctive specific characters.

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NOTES ON SOME NEMATODES IN THE MUSEUM OF THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE.—II

BY

E. L. TAYLOR

(*Received for publication* 28 *December*, 1924)

SPIRONOURA CONGOLENSE, n.sp.

Material :—Specimens collected in the Congo from a fish.

This worm presents all the characters typical of the genus with the exception of the arrangement of caudal papillae in the male, which appears to be somewhat variable in this species.

The body tapers towards the extremities in both sexes and is covered with exceedingly fine cross striations. The head (figs. 1 and 2) presents the usual globular outline and is followed by a well-marked neck. The male measures 13 to 17 mm. in length, by 0·45 to 0·72 mm. in greatest diameter; the head has a diameter of 0·183 to 0·21 mm., the small cervical papillae are placed 1·47 and

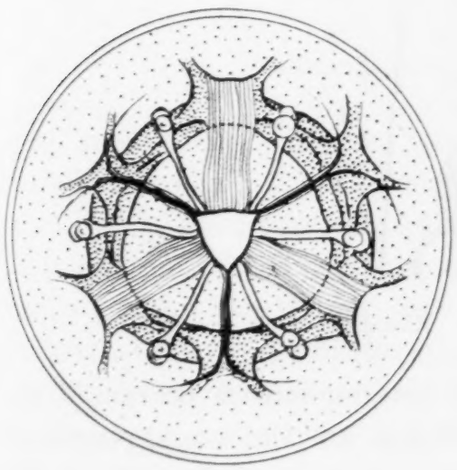

Fig. 1. *Spironoura congolense*, n.sp. Head, anterior view. × 250.

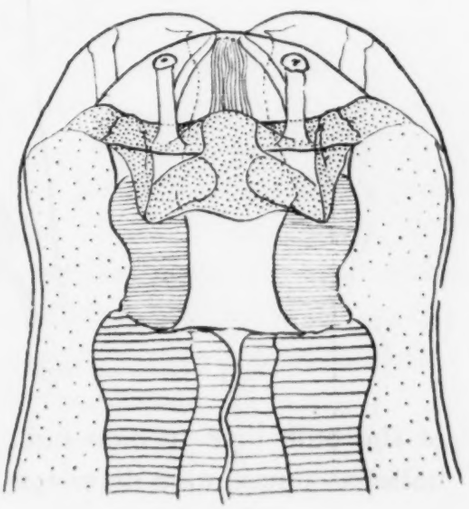

Fig. 2. *Spironoura congolense*, n.sp. Head, dorsal view. × 250.

1·72 mm. from the anterior extremity and the excretory pore 1·95 to 2·1 mm. from the same point. The pharynx joins the second part of the oesophagus at a point 0·1 to 0·116 mm. from the anterior extremity, from which point the oesophagus continues as a cylindrical muscular tube to the double bulb at its extremity; the complete length of the oesophagus (fig. 3) is 2·85 to 3·00 mm. and the length

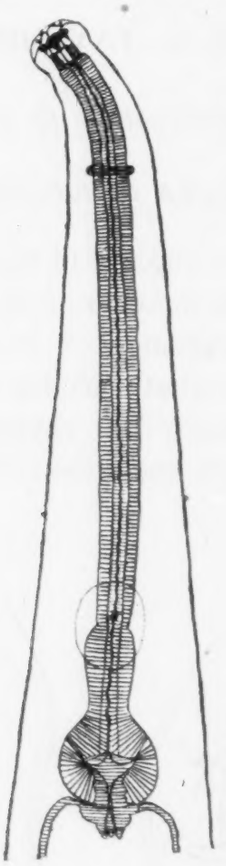

Fig. 3. *Spironoura congolense*, n.sp. Anterior extremity. × 37.

of the bulb 0·58 to 0·60 mm.; the anterior narrow portion of the bulb has a maximum diameter of 0·18 mm. and the second portion of 0·36 mm. The nerve ring surrounds the second part of the oesophagus at a point 0·52 to 0·66 mm. from the anterior extremity. The caudal extremity (fig. 4) is ventrally curved and terminates in a sharp point; the special caudal muscles are well developed and continued for a distance of about 3·3 mm. forward along the ventral aspect, but the fan-like formation of muscular fibres forming the pseudo-sucker, seen in some species, is entirely unrepresented. The

spicules are short, measuring only 0·51 to 0·6 mm. in length, and having a maximum diameter of 0·073 to 0·10 mm. The gubernaculum is a distinct and well chitinised organ. The preanal papillae number three pairs and are very small, the unpaired preanal papilla is present. The postanal papillae vary from seven to nine in number : of the four males present two showed eight postanal papillae on the right-hand side and seven on the left, one showed nine on the right and seven on the left, and the remaining specimen showed eight on either side. A further variation from the generic type is

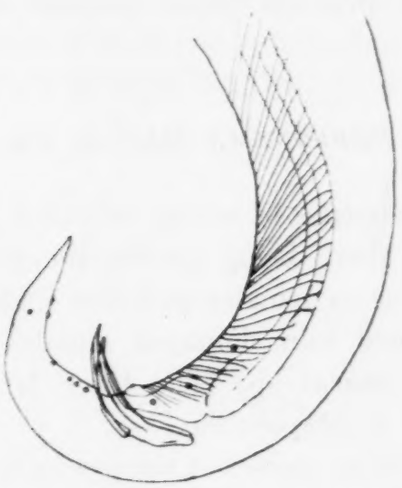

Fig. 4. *Spironoura congolense*, n.sp. Male. Caudal extremity. × 27.

seen in the absence in some specimens of the second lateral postanal papilla—Baylis (1922), points out the constant presence in this genus of at least two pairs of lateral papillae postanal—two of the four males examined had only one pair of these papillae as seen in the figure ; when the second pair was present it was placed half-way between the arc seen in the figure and the cloaca.

The female measures 13 to 22 mm. in length by 0·45 to 0·795 mm. at its greatest diameter ; the head is 0·2 to 0·232 mm. in width, the distance from the anterior extremity of the excretory pore is 1·95 to 2·55 mm. and to the cervical papillae 1·65 to 1·85 mm. The oesophagus has a complete length of 2·55 to 3·06 mm., the bulb measures 0·57 to 0·69 mm. in length and 0·3 to 0·36 in diameter at the second portion. The distance from the caudal extremity to the anus is 1·125 to 1·375 mm., the vulva is placed posterior to the middle of the body, being 5·25 to 10 mm. from the posterior extremity,

the vagina has a length of 0·6 to 1·2 mm. and is directed antero-dorsally ; it divides into the two divergent branches which describe the usual loops before reaching the ovaries. The eggs are large and filled with a granular mass when laid ; they measure 106 by 73 μ.

In general form and measurement this worm closely resembles *S. barbi* (Baylis, 1922), but differs in the arrangement of the papillae on the caudal extremity of the male, in the length of spicules and in the absence of the pseudo-sucker. *S. barbi* has spicules about twice the length of the spicules of this species, has a well-developed pseudo-sucker, and presents three preanal and seven postanal papillae.

HABRONEMA MAGNA, n.sp.

Material :—Two bottles of worms collected from the air-sacs of *Trachurus declivis*, there being twelve females and eight males in one bottle and seven females and five males in the other. A third bottle contained four damaged females collected from the sub-peritoneum of *Sparus* sp. The three lots were collected in Australia by Dr. P. A. Maplestone.

Compared with other species of the genus *Habronema* this worm is large, measuring up to 94 mm. in length and 1·2 mm. in width. The body is of a dirty yellowish-white colour and of a fairly even thickness throughout its length, tapering a little towards the two extremities. In either sex the cuticle in the anterior part of the body shows a rather coarse transverse striation, but posteriorly the markings are different in the two sexes as described below. Cuticular alae are bilateral in both sexes and in cross section are seen to be as thick as broad. The head may be described as having two large lateral lips and two smaller median lips continuous with the lateral lips by means of a cuticular fold (figs. 5, 6 and 7). Seen anteriorly the two lateral lips appear as triangular pieces base to base. The median lips are much smaller structures, and in viewing the head dorsally appear as thickenings in the level fold of cuticle joining the lateral lips : viewing the head laterally these median lips are seen to have considerable thickness, and an anterior view of the head shows them to project in a wedge-shaped manner into the space between the outer edges of the two lateral lips.

Gendre (1923) makes a general distinction between the type of head seen in species belonging to the genus *Habronema* from birds and from mammals. It is only a very general difference and cannot be strictly adhered to, but he points out that species parasitic in birds have large triangular lateral lips with a broad extremity and narrow base, and two median lips on a broad base, each composed of two lateral globular masses, with a median conical piece, and carrying the two papillae, on the contrary the type parasitic in mammals presents lateral lips of a more quadrangular shape, joined on either side merely by a cuticular fold in place of the two median lips. The type of head seen in the species here described may be regarded as intermediate between the two; the lateral lips show the

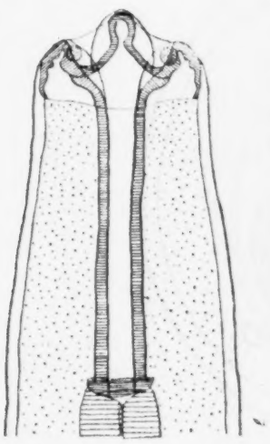

FIG. 5. *Habronema magna*, n.sp. Head, lateral view. × 125.

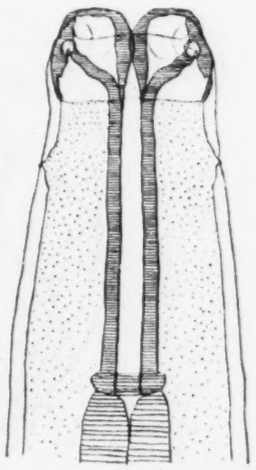

FIG. 6. *Habronema magna*, n.sp. Head, dorsal view. × 125.

triangular form of the avian type, while the median lips, although showing the wedge-shaped point are without the two lateral globular masses and are not separated by any dividing cleft from the lateral lips, in which two points the head resembles the mammalian type.

The whole head structure is strengthened with chitin which forms an outer subcuticular capsule as well as lining the mouth parts, where it is continuous with the thick chitinous wall of the pharynx. There are four, large, flat, submedian papillae, placed just below the margin of the cuticular folds between the lateral and median lips. The cervical papillae are small and situated far forward, one-third the distance down the pharyngeal portion of the

body. The pharynx is thick-walled, long and cylindrical in form. The oesophagus is long and divided into two portions ; the anterior muscular portion is narrow and about one-third the length of the second part ; at a short distance from its anterior end it carries the nerve ring. After the junction of muscular and glandular portions the oesophagus rapidly widens to twice its former diameter and from this point it continues at an even width to its junction with the intestine.

The male measures 23·25 by 0·45 mm. to 25 by 0·6 mm. ; the head has a diameter of 0·15 to 0·17 mm., the pharynx has a diameter of 0·04 to 0·043 mm. and terminates a distance of 0·33 to 0·345 mm.

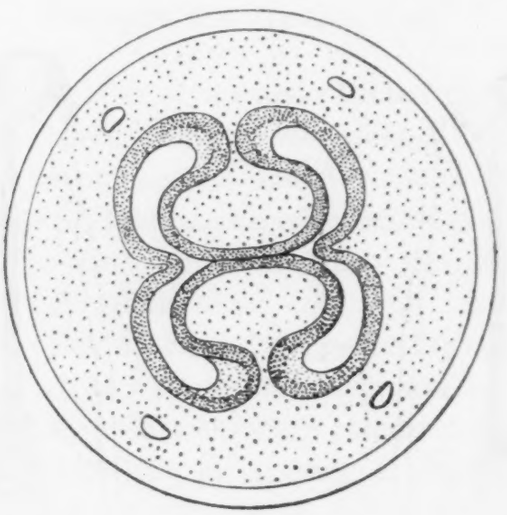

Fig. 7. *Habronema magna*, n.sp. Head, anterior view. × 250.

from the anterior extremity. Cervical papillae are situated at a distance of 0·133 mm. and the excretory pore 0·70 to 0·75 mm. from the same point. The length of the first part of the oesophagus is 1·5 mm. and of the second part 3·9 mm. ; the nerve ring surrounds the first portion at a distance of about 0·43 mm. from the anterior extremity. The distance between the cuticular striations increases from about 5·5μ at the anterior end to 25μ near the caudal extremity. These transverse striations are only continued to the caudal extremity on the dorsal side of the lateral alae ; the ventral aspect of the worm for the posterior 9 mm. of its length presents a series of parallel longitudinal folds in the cuticle, each about 30μ wide, these are

continued up to a point just anterior to the cloaca. The caudal extremity is spirally coiled and describes two or three complete turns. Towards the cloaca the lateral alae widen in each dimension, reaching a maximum width just in front of this orifice where they are broad and semi-cylindrical in shape; posterior to this point they diminish in size to the extremity of the tail. The pedunculated papillae number eight pairs (figs. 8 and 9); there are four large pedunculated preanal pairs, the posterior three of which are in a line subventrally placed, while the anterior pair is more laterally placed, a little in advance of the second papilla. Posterior to the anus and a short

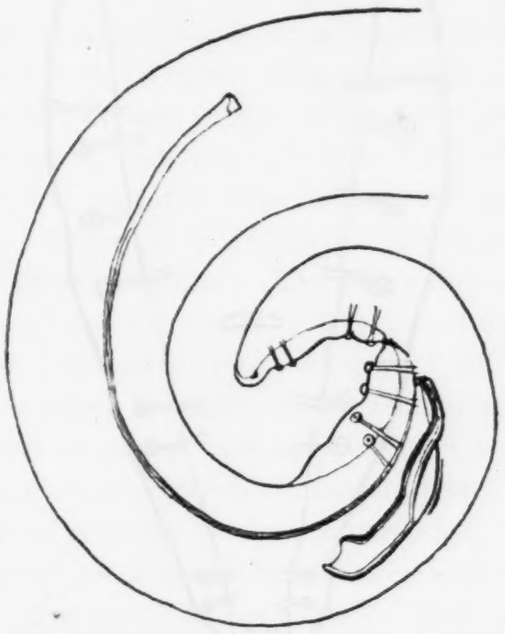

FIG. 8. *Habronema magna*, n.sp. Caudal extremity of male, lateral view. × 55.

distance behind, are two pairs of similar large pedunculated papillae sub-ventrally placed, while two much smaller pedunculated pairs occupy a subventral position nearer the extremity. Near to the extreme end and ventrally placed are two broad sessile papillae, each carrying three points. The spicules are very unequal in size, but differ from those of other species of *Habronema* in that the long, delicate spicule is placed on the right side of the worm, while the short one is the left spicule. The long spicule varies in length from 1·7 to 1·8 mm. and in average width of shaft from ·013 to ·023 mm., being broader at the proximal end and tapering to a very fine point at the

extremity ; the spicule appears to carry a lateral flange in its posterior three-quarters. The short left spicule is very short and of a peculiar shape ; the proximal end is in the form of a wide bulb and is bent ventrally ; this is followed by a stout cylindrical shaft which leads to a narrow portion, that describes a gradual dorsal curve followed by a decided ventral bend ; a short distance from the extremity it bends forwards to the side and terminates in two divergent points ;

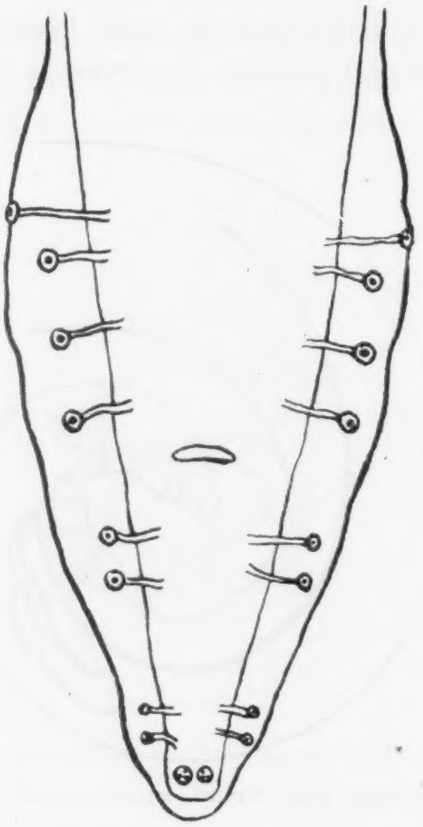

FIG. 9. *Habronema magna*, n.sp. Caudal extremity of male, ventral view. × 80.

there is also a keel-like piece which, commencing dorsally in the middle of the shaft, winds round the outer side to terminate ventrally near the extremity ; this spicule varies in length from 0·39 to 0·45 mm. and in greatest thickness from 0·053 to 0·063 mm. The gubernaculum is a small, well-defined piece about 0·093 mm. long and lies immediately behind the shaft of the short spicule. The tail measures 0·27 to 0·31 mm. from cloaca to extremity.

The female measures 19 by 0·30 to 94 by 1·2 mm. The diameter of the head is 0·13 to 0·285 mm., the cervical papillae are placed at

0·1 to 0·166 mm. from the anterior extremity and the excretory pore is 0·52 to 1·05 mm. from the same point. The cuticle in the anterior part of the body is striated at intervals of about 4μ near the head, increasing to intervals of 22μ just anterior to the vulva; here the narrow grooves which cross the raised portion between the striations are more in evidence than they are near the head and the raised portion is seen to be composed of numerous more or less oblong shaped elements; posterior to the vagina these elements rapidly increase in size and near the caudal extremity the striations are seen to be at a distance of 99μ apart, rather irregular in appearance and the raised intermediate portion composed of projecting pieces of varying shape and size, roughly twice as long as broad. The lateral alae are stout structures commencing about 0·75 mm. from the head in a mature female and projecting at their maximum width a distance of 0·076 mm.; they are almost as thick as they are broad and have a rounded, striated edge; they are prolonged to the caudal extremity, where they gradually diminish in width, and present a rather broken outline. The pharynx has a diameter of 0·025 and 0·066 mm. and terminates a distance of 0·226 to 0·45 mm. from the anterior extremity. The first part of the oesophagus measures 1·005 to 2·4 mm. in length and the second part 3·3 to 7·5 mm. The vulva (fig. 10) is placed ventrally about the junction of the anterior and middle third of the body length, being 8·5 to 23·5 mm. from the anterior extremity; it is surrounded by a prominent muscular ring which in a small female measured 0·18 mm. deep and 0·28 mm. in diameter. In the gravid female the anterior and posterior parts of this ring meet one another to form two prominent muscular lips. The vagina opens on the inner side of the anterior lip, from which place it may be seen to take an immediate turn backwards. After leaving this muscular ring, the vagina is continued backward as a long, straight muscular tube 0·04 to 0·07 mm. in diameter and up to 22 mm. long; at its extremity it divides into the two divergent branches of the uterus. In one immature female the vagina was found to run back a distance of 0·9 mm., then bend forwards to a point 1·5 mm. in front of the vulva, then double back again for a distance of 0·75 mm. where it divided. The caudal extremity of the female is a short, blunt cone and is usually bent dorsally; the anus is about 0·1 to 0·21 mm. from the extremity.

The eggs are about 37 by 23μ in size, thick shelled and have a small 'button' arrangement at either end, from each of which proceed two very delicate flagella each of about the same length as the egg. The contents of the egg are segmented when laid.

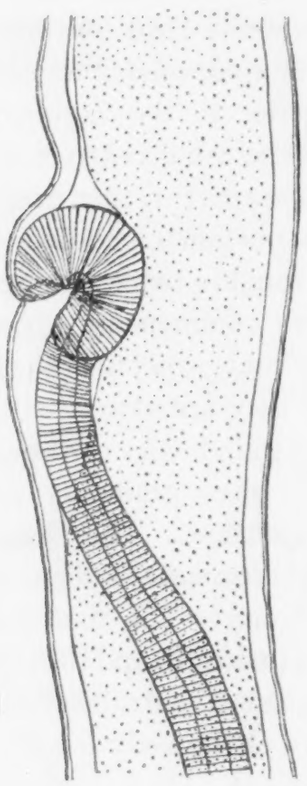

FIG. 10. *Habronema magna*, n.sp. Genital opening of female. × 190.

CONTRACAECUM CLAVATUM (Rud., 1809)

Material :—Seven bottles of worms from *Gadus morrhua*, two from *G. merlangus*, one from *Solea* sp., one from *Raja* sp. and one from *Belone belone*.

Most of the bottles contained a large number of worms in a good state of preservation.

Two clearly related species of *Contracaecum* have been described in these hosts. *Contracaecum clavatum* (Rud., 1809) and *Contracaecum pedum* (Deslgch, 1824), the only difference apart from a slight difference in total length being in the length of spicules; the male *C. pedum* is described as 32 mm. long, with spicules 2·4 mm.

long ; and *C. clavatum* as 33 and 46 mm. long with spicules measuring 1·25 mm. This difference in spicule length is quite a considerable one and when worms were found in the material at hand showing this variation, it was at first thought that two species were present ; on making further examinations, however, and taking a large number of measurements of worms picked at random from various bottles, it was found that only one species was represented. Without any reference to size or relative maturity of worm in some forty specimens measured, the ratio of spicule length to total length showed an even variation between the extremes of $\frac{1}{9{\cdot}5}$ to $\frac{1}{27}$. In view of the absence of any further difference between the two species, it seems that the two worms are synonymous and refer to a species which shows a rather remarkable variability of spicule length, so that *C. pedum* (Deslgch, 1824), falls as a synonym of *C. clavatum* (Rud., 1809).

PROCAMALLANUS LAEVICONCHUS (Wedl, 1862)

Material :—Four females collected from a silurid fish in the Congo.

These specimens conformed in every way to Baylis's (1923) description, but as there do not seem to be in existence any clear drawings of the rather peculiar mouth capsule, it has been thought advisable to produce some from the well-preserved specimens available (figs. 11 and 12).

The mouth parts may be described as follows :—The buccal capsule is deep and of a dark brown colour and has a particularly thick wall at the bottom where it joins the oesophagus. At the anterior opening of this capsule are six inwardly curving plates directed forward ; these plates have rounded extremities and are adjacent towards their free ends, but at their bases are separated by spaces in the chitinous wall of the capsule ; the spaces are both broader and deeper between the two subventral and between the two subdorsal plates, so that although the mouth is not actually in the form of a dorso-ventral slit—which is the type met with in the family *Camallanidae*—it still has a bilateral symmetry which approaches the type. Anteriorly the mouth is bounded by an

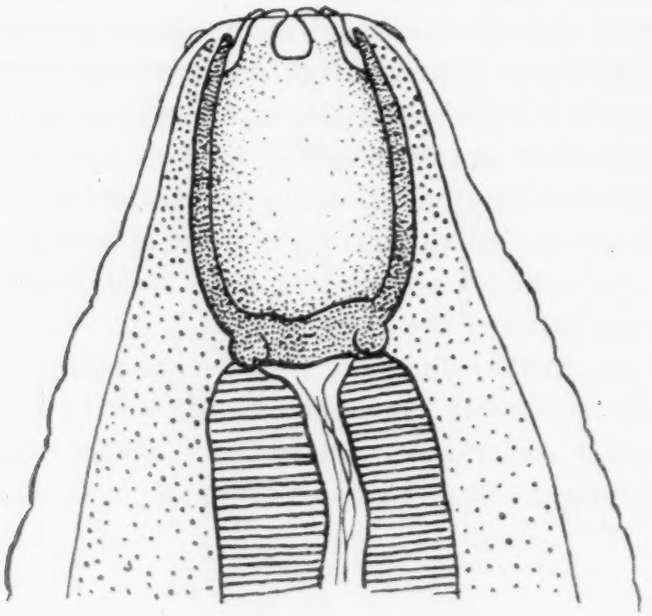

FIG. 11. *Procamallanus laeviconchus.* Head, dorsal view. × 500.

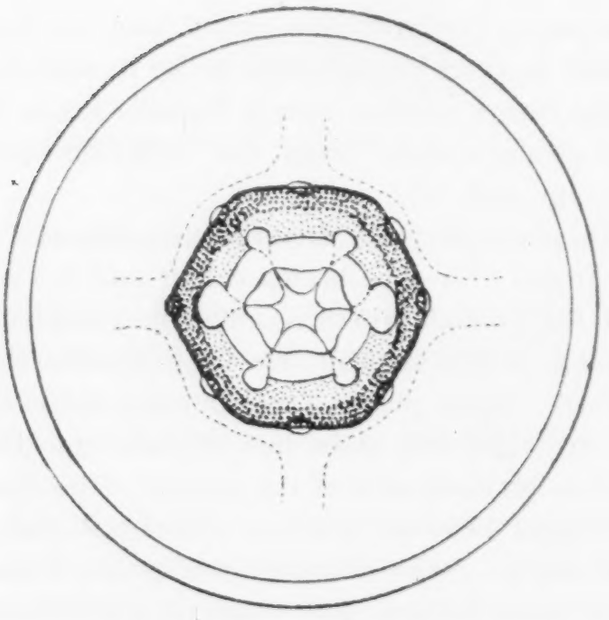

FIG. 12. *Procamallanus laeviconchus.* Anterior view. × 500.

external membrane which is merely a continuation of the integuments of the body ; the orifice formed is somewhat hexagonal, corresponding with the hexagonal shape of the buccal capsule. Eight head papillae are present, one dorsal, one ventral, two subdorsal, two subventral and two lateral.

ECHINOCEPHALUS SOUTHWELLI, Baylis, 1920

Material :—Male and female specimens collected from *Urogymnus* sp. in Ceylon.

ECHINOCEPHALUS SPINOSISSIMUS (v. Linstow, 1905)

Material :—Male and female specimens collected from *Trygon sephen*. Pearl Banks, Ceylon.

PROLEPTUS OBTUSUS, Duj., 1845

Material :—Numerous specimens collected from *Acanthias vulgaris, Scyllium caniculum* in Ceylon, and *Coronilla scillicola* in South Africa.

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ON THE GENUS *TETRACAMPOS*, WEDL, 1861

BY

T. SOUTHWELL

(*Received for publication* 17 *January*, 1925)

Genus *Tetracampos*, Wedl, 1861.

SYNONYMS :—*Ophryocotyle*, Southwell, 1913.
Gangesia, Woodland, 1924.

In 1861, Wedl described a cestode from a fresh-water fish under the name *Tetracampos ciliotheca* ; but he gave no definition of the new genus which he erected. Braun (1900) described the characters of the genus as follows :—

Head with four bothridia. Rostellum in the form of a cupola. On this rostellum there are four groups of nine hooks. The hooks are of unequal length, slightly curved, ending in a claw ; the longest hook is in the middle, the shortest hooks are at the sides of each group. Neck of average length ; four excretory canals to each segment. Genital pores on the flat sides. Egg thin shelled, containing a ciliated onchosphere.'

Type species :—*Tetracampos ciliotheca*, Wedl, 1861, from *Heterobranchus anguillaris*.

The following is an abstract of Wedl's description of the worm :—

Tetracampos ciliotheca (fig. 1).

Specimens of the above were found in the mucus from the intestine of *Heterobranchus anguillaris* (from the Nile) immediately below the stomach. They are delicate, thread-like worms 10 mm. to 15 mm. in length. The button-like head measures 0·2 mm. in breadth and is of a remarkable structure, recalling, by virtue of its 'lobes' that of *Tetrabothrium*. Each 'lobe' consists of parenchyma and is thin-walled and contractile, projecting as a flat disc. Anteriorly these lobes (bothridia of van Beneden) approach one another and encircle (surround) a projecting cupola-shaped armed papilla. The hooks, which may be differentiated into a long stalk with a short, slightly curved, pointed, sickle-shaped process or continuation, form four groups and are not arranged in circles or rows as in species of the genus *Taenia*. Each group generally consists of nine hooks, with the longest hook in the middle, and the shortest hooks at the outside of the group. A line drawn through the points of the hooks would describe an arc.

For a short distance behind the head, the segments are delicate, transparent, rounded off laterally, and connected to one another by well-developed longitudinal muscles. Two pairs of parallel vessels with transverse anastomosing branches run through the segments, and, in the head region, divide up into a complex network.

The last segment is cone-shaped (strobiliform) and possesses a distinct so-called 'porus excretorius.'

The genital pores are situated on the middle of the flat surface of each sexually mature segment. The eggs enclosing the hexacanth embryo are peculiar. Eggs were taken from the last segments and kept under observation, and it was noticed, after the external egg-skin had burst open, that the internal covering was furnished with comparatively long cilia, which were in rapid movement; these produced not only a rotatory but also a forward movement.

(Wedl at first doubted this phenomenon, but after observing many eggs, satisfied himself that the embryophore was ciliated.)

The anatomy of the worm was not described, but the essential features of the species are the presence of an armed rostellum and the fact that the embryophore is ciliated. It is impossible to decide from Wedl's figure and descriptions whether the so-called 'bothridia' are really outgrowths from the head, or whether they are true acetabula.

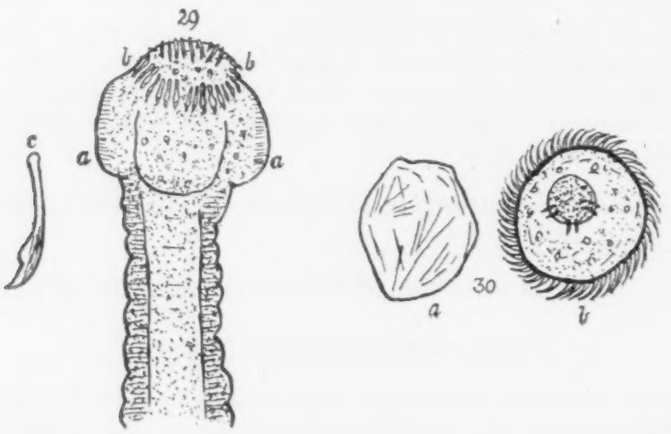

FIG. 1. *Tetracampos ciliotheca*, after Wedl. Magnification unknown.
(29) Head. *a.a.*—bothridia; *b.b.*—the four groups of hooks; *c.*—an isolated hook;
(30) *a.*—external egg-shell; *b.*—internal ciliated egg-shell with embryo.

Wedl states that the genital pores are situated on the flat surface of the worm (i.e., ventrally), but it appears probable that the sexual apertures are situated laterally, and that the apertures to which he refers are secondary pores, caused by the dehiscence of the gravid uterus.

It is to be noticed that the worm was obtained from the intestine of a fresh-water cat-fish (*Heterobranchus anguillaris*). The adult

cestode parasites most common in fresh-water fishes belong to the genus *Proteocephalus* (Weinland, 1858) La Rue, 1914.

La Rue (1914) ascribed the following characters to the family PROTEOCEPHALIDAE :—

'Heads small. Suckers sessile and without accessory areola. Fifth sucker functional, vestigial, or lacking. No rostellum. Genital organs as in other Tetraphyllideans. Genital pores marginal, irregularly alternating. Vitellaria lateral, follicular, follicles closely grouped about a central conducting tubule. Ovary bilobed, posterior. Oöcapt, oötype, shell gland, uterine passage present. Uterus with lateral outpocketings and one or more preformed, ventral, uterine openings. Vitellaria, testes, ovary and uterus *within* the inner longitudinal muscle sheath.

'Habitat.—In fresh-water fish, amphibia, and aquatic reptiles.'

La Rue defined the genus *Proteocephalus* as follows :—

'With the characters of the family.

'Head globose or conical, flattened dorso-ventrally. No rostellum. No spines or hooks. No fold of tissue encircling base of head or enfolding suckers. Suckers circular or oval. Fifth sucker functional or vestigial, rarely lacking. Testes in a broad field between vitellaria. Parenchyma with close meshes. Musculature well developed. Eggs with three membranes. Habitat :—In fresh-water fish.'

It is clear that, owing to the presence of an armed rostellum, *Tetracampos ciliotheca* cannot be referred to the genus *Proteocephalus* as defined above, but, as other authors have since recorded similar worms with an armed rostellum and possessing an internal anatomy typical of the genus *Proteocephalus*, it is most probable that the internal anatomy of Wedl's specimen was also typical of the genus *Proteocephalus*.

As a result, it is necessary to emend the characters of the family so as to include within it a genus with an armed head.

The writer in 1913 described as follows a worm which should clearly be referred to Wedl's genus *Tetracampos* :—

Ophryocotyle bengalensis, Southwell, 1913 (fig. 2)

'Over sixty specimens of this worm were obtained from the intestine of *Ophiocephalus striatus*, and a few were also obtained from the intestine of *Labeo rohita*. Both fish were caught at Berhampur Court, Bengal, in a fresh-water tank. This genus of tapeworm usually occurs in birds, and considerable interest attaches to the presence of these adult forms in Teleosts. The average length of the worms was 7·5 mm. Greatest breadth (at posterior end), 0·8 mm. These latter segments were from four to five times broader than long. The head consists of four cup-shaped suckers, directed slightly forward. Anteriorly the head terminates in an umbrella-shaped protrusible rostral disc whose circumference is armed with a large number of hooks arranged in two rows. The exact number could not be determined,

as, in removing the parasites from the intestine of the fish, many of the hooks had been torn away. The exact number counted in three specimens is given in the following table :—

(i) One row of twenty-five hooks.
(ii) Two rows with a total of fifty-three hooks.
(iii) Two rows with a total of fifty-two hooks.

'The hooks appear to be all similar. They have broad bases and are sharply recurved in profile. Viewed end on, they appear elongated.

'The suckers are armed with exceedingly minute spines which appear to be limited to their anterior borders. The head measures about 0·5 mm. broad. The neck is fairly long, measuring 2·7 mm. Dots of black pigment are scattered about over the whole worm. The first proglottides are exceedingly shallow, and *all* proglottides are broader than long. The lateral margins are wrinkled in such a way that in young specimens the true strobilization can only be determined under a lens. The genital apertures are lateral and are almost all on one side.

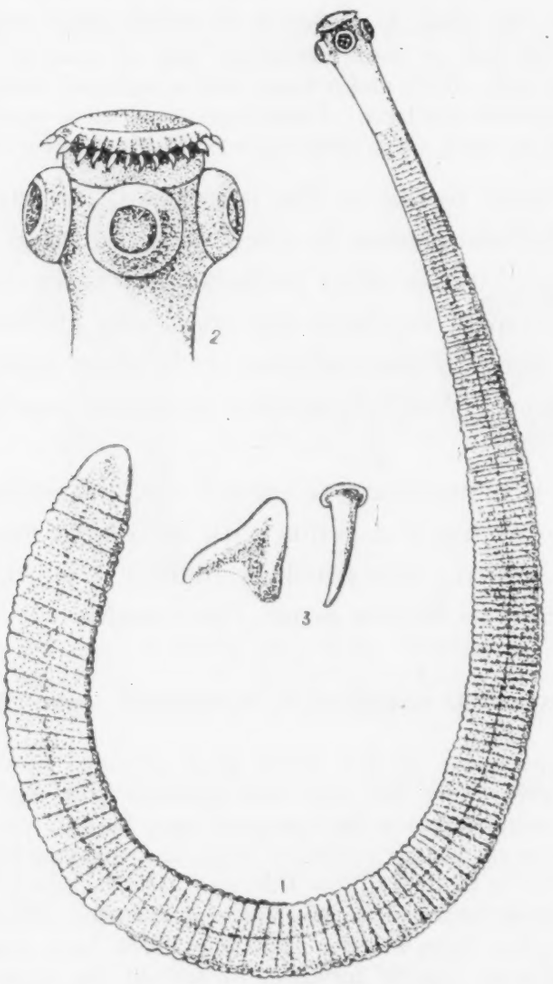

FIG. 2. *Tetracampos bengalensis* (Southwell) = *Gangesia wallago*, Woodland. (1) Entire worm. × about 30. (2) Head. × about 180. (3) Hooks greatly enlarged. After Southwell.

'The uterus appears to be made up of a number of rounded egg capsules scattered about the proglottid.

'Habitat :—The intestines of *Labeo rohita* and *Ophiocephalus striatus*, Berhampur Court, Bengal, June, 1912. About sixty specimens.

'Amongst the worms just described were two large specimens measuring 27 mm. and 22 mm. respectively. They differ from the smaller forms only in having the neck very much shorter and in being much larger. Two rows of about fifty hooks were counted round the circumference of the rostral disc.'

A re-examination of a single specimen of this species (the only one now in the writer's possession) has brought to light the fact that the internal anatomy of the worm is exactly similar to that found in species of the genus *Proteocephalus*.

The hooks on the head are all alike and are in a *single* crown as originally figured; they measure about 35 μ. The uterus is rudimentary and does not contain egg capsules as suggested in the above description.

Although the species is clearly to be referred to the genus *Tetracampos* it is undoubtedly different from *T. ciliotheca*; for, in *O. bengalensis*, the rostellum is armed with a crown of hooks, all of which are similar, whilst in *T. ciliotheca* the rostellum is armed with four groups of hooks which are not uniform in size.

Woodland (1924) has just described two species of cestodes, viz., *Gangesia wallago* and *G. macrones* from India, obtained from the intestines of *Wallago attu* and *Macrones seenghala* respectively, for which he erects a new genus with the following characters :—

'*Gangesia* :—with the characters of the family PROTEOCEPHALIDAE but emended to include forms with armed rostella. With a scolex possessing a globose muscular rostellum armed or unarmed, and no fifth sucker. The suckers may or may not bear spinelets. Testes in a single broad field between vitellaria. Eggs with three membranes. Habitat :—In fresh-water fish.'

Woodland described the two species as follows :—

G. wallago, Woodland, 1924 (fig. 3).

'Length of strobila usually not exceeding 40 mm., with a maximum breadth of about 1·5 mm. Proglottides numerous, well over 100 in number in mature forms, narrow antero-posteriorly in front but square or elongated posteriorly. Segmentation distinct. Scolex 0·166 to 0·232 mm. long and 0·298 to 0·448 mm. broad. Suckers with projecting edges, 0·120 to 0·172 mm. broad, and in part bearing numerous closely-set spinelets. The globular rostellum bears a single circle of hooklets, all of one kind, 29·28 to 43·92 microns long and twenty-eight to forty-two in number. A very short neck is present, but is only visible in specimens with the scolex torn off or in flattened specimens, and gradually increases in diameter up to the first traces of segmentation. Genitalia like those of *Proteocephalus*. Uterine diverticula twenty to twenty-eight in number. Testes over 100 in number,

65·8 to 109·8 microns in length and maximum breadth of 28 microns. Genital openings lie a little in front of the middle transverse line of the proglottid, and the cirrus sac and vaginal openings vary as to which is anterior. The uncontracted cirrus sac extends over about one-third of the distance across the proglottis. Eggs provided with three membranes, the outermost being 91·5 to 98·8 microns in diameter and the spherical embryo measuring 18·30 to 21·96 microns. Habitat :—intestine of *Wallago attu* Bleek (and probably *Ophiocephalus striatus* and *Labeo rohita*), rivers of India.'

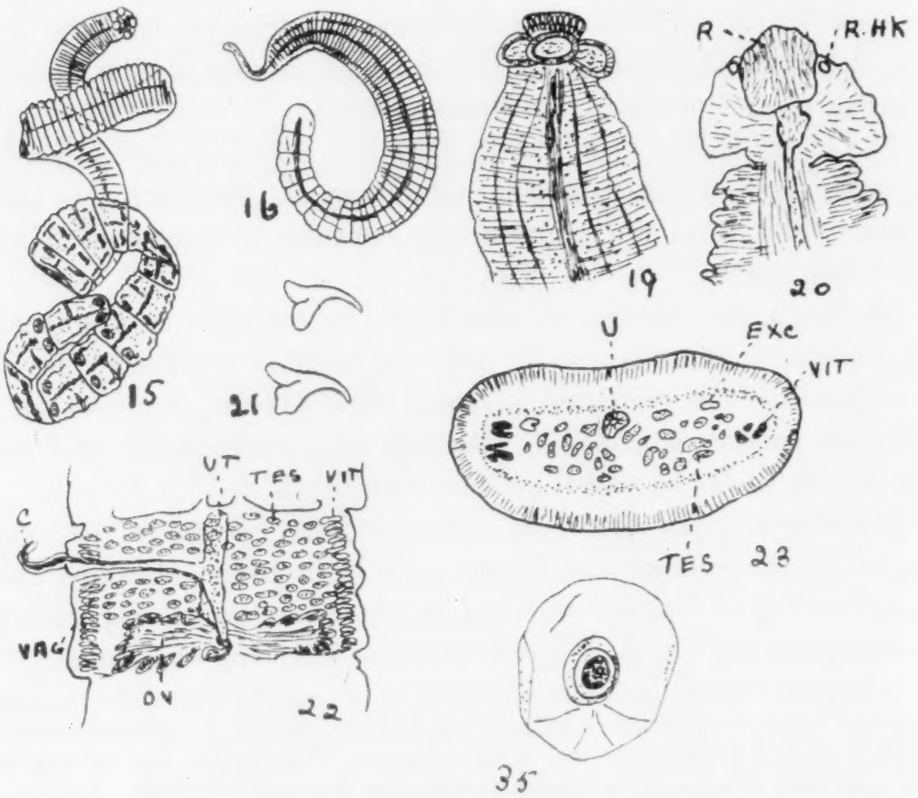

FIG. 3. *Tetracampos bengalensis* (Southwell, 1913)=*Gangesia wallago* (Woodland, 1924). (15) A mature worm (actually about 12 mm. long) with scolex. × 12. (16) A small worm (actually about 4 mm. long) with scolex torn off and showing the drawn-out short neck. × 12. (19) Scolex with rostellum and suckers protruded and a slight indication of the short contracted neck. × 30. (20) Longitudinal section through scolex to show the limits of the muscular rostellum. × 87·5. (21) Two hooks from the rostellum. × 260. (22) Flattened mature proglottid. × 27·5. (23) Transverse section through a mature proglottid just behind the level of the cirrus sac. × 56. (35) Egg (fully developed) from contents of the fish intestine. × 180. *r.*—rostellum; *r.h.k.*—rostellar hooks; *u.*—uterus; *exc.*—excretory canal; *vit.*—vitellaria; *tes.*—testes; *vag.*—vagina; *ut.*—uterus; *ov.*—ovary; *c.*—cirrus. After Woodland.

G. macrones, Woodland, 1924 (fig. 4).

'Length of strobila does not exceed 60 mm. in length, with a maximum breadth of about 1·2 mm. Proglottides numerous, between 150 and 200 in number in mature worms, very narrow antero-posteriorly in front but square or elongated posteriorly. Segmentation distinct. Scolex measures about 109 microns long and 193 microns broad. Suckers small and thick-walled, about 67 microns broad

and bearing numerous closely-set spinelets on their upper edges and adjacent internal surfaces. The globular rostellum (about 109 microns in diameter) bears a single circle of hooks, of two kinds, large (11·0 to 14·6 microns long) and small (about 6 microns long) alternating. Neck absent. Genitalia like those of *Proteocephalus*. Uterine diverticula twenty to thirty in number. Testes over 100 in number. The genital apertures lie in front of the middle transverse line of the proglottid and usually the cirrus sac opening is anterior to the vaginal but the reverse condition also occurs. The uncontracted cirrus sac in flattened specimens extends over only from one-sixth to one-quarter of the breadth of the proglottis. Habitat :— Intestine of *Macrones seenghala* Sykes, from rivers of India.'

FIG. 4. *Tetracampos macrones* (Woodland, 1924)=*Gangesia macrones*, Woodland, 1924. (26) Scolex viewed in optical section. × 180. (27) Rostellar hook. × 530. (28) Mature flattened proglottids (both cirrus sacs have been drawn too long, judging from later measurements). × 17·5. (30) Sketch of a gravid proglottid with outlines of fully developed uterine diverticula (full of eggs in actual preparations). The central stem of the uterus is considerably flattened. × 17·5. After Woodland.

As all the preceding species possess an armed rostellum and are found in fresh-water cat-fish there can be no doubt that they are all to be referred to Wedl's genus *Tetracampos*, which, as Braun's description is inadequate, is now redefined as follows:—

PROTEOCEPHALIDAE. *Body segmented; head with four suckers, and armed with hooks. Internal anatomy as in the genus Proteocephalus, La Rue. Genital pores marginal and irregularly alternate. Adults parasitic in fresh-water fishes.*

Woodland proposed emending the characters of the Order *Tetraphyllidea*, Lühe, 1910, and of the Family PROTEOCEPHALIDAE, La Rue, 1914, in order to include the forms which possess an armed rostellum. Apparently it did not occur to him that cestodes whose heads are armed with four suckers probably belong to the Order *Cyclophyllidea*.

Prior to the appearance of Woodland's paper, the writer had already made an exhaustive study of the worms included in the Order *Tetraphyllidea* and had arrived at the conclusion that the order should be limited so as to include only species in which the head bears four bothridia (lappet-like outgrowths from the head).

The family PROTEOCEPHALIDAE, which possesses four suckers, or acetabula, falls naturally into the *Cyclophyllidea*, but differs from most other families of that order in having numerous vitelline glands situated laterally, instead of being condensed into a single mass in the vicinity of the ovary.

The *Cyclophyllidea* were accordingly split up by the writer into two sub-orders, viz.: (1) the *Univitellata*, comprising all those forms with four suckers, and in which the vitelline glands are condensed into a single mass; and (2) the *Multivitellata*, comprising all other forms with four suckers, in which the vitelline glands are either situated laterally or extend over the dorsal and ventral surface of the worm.

Wedl's genus *Tetracampos* is referred to the sub-order *Multivitellata*. As Braun, however, stated that the head was armed with four bothridia, the writer in his Monograph dealt with it under the Family ONCHOBOTHRIIDAE. The genus clearly belongs to the Family PROTEOCEPHALIDAE, La Rue, 1914, which is emended accordingly as follows:—

Body segmented; head small, bearing four suckers (acetabula) and either armed or unarmed. Fifth sucker functional, vestigial or

lacking. Genital pores marginal and irregularly alternate. Vitellaria lateral or extending over the dorsal and ventral surfaces; Uterus with lateral outpocketings and with one, or more, preformed, ventral uterine openings. Habitat :—In fresh-water fish, amphibia and aquatic reptiles.

The genus *Tetracampos*, Wedl, 1861, is thus referred to the Family PROTEOCEPHALIDAE (La Rue, 1914) emended; this family is placed in the sub-order *Multivitellata*, Southwell, 1925. of the Order *Cyclophyllidea*, Southwell, 1925.

The genus at present contains three species, viz. :—

(1) *Tetracampos ciliotheca*, Wedl, 1861.

(2) *Tetracampos bengalensis* (Southwell, 1913).

SYNONYMS :—*Ophryocotyle bengalensis*, Southwell, 1913.
Gangesia wallago, Woodland, 1924.

(3) *Tetracampos macrones* (Woodland, 1924).

SYNONYM :—*Gangesia macrones*, Woodland, 1924.

Woodland points out that the specimens of *O. bengalensis* Southwell, 1913, are 'almost certainly examples of *G. wallago*,' but

'I think Southwell's wholly insufficient description of his *Ophryocotyle bengalensis* justifies me in not adopting his specific name for my type species of *Gangesia*. Only an adequate statement of distinctive characters can justify claim to priority.'

As the description of *O. bengalensis* was sufficient to enable Woodland to state that his *G. wallago* is almost certainly the same, there is obviously no justification whatever for burdening the literature with other names. Under the ordinary rules of nomenclature, *Gangesia wallago* becomes a synonym of *Tetracampos bengalensis* (Southwell, 1913).

The writer is indebted to the Editor of *Parasitology* for permission to reproduce the descriptions and figures of *Gangesia wallago* and *G. macrones*.

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ON THE BIONOMICS OF *HIPPOBOSCA EQUINA*

BY

J. ISGAER ROBERTS, B.Sc. (Wales)

(Laboratory of Agricultural Zoology—Department of Agriculture—The University, Bangor)

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Distribution in Wales

Hippobosca equina is very localised in its habits, being more abundant on the Continent than in Great Britain; records of infestation of horses and cattle are found in most European countries, and also from Palestine, where it is extremely common on horses and dogs during spring and summer (Buxton, 1924). In Britain, the New Forest in Hampshire and certain of the sheltered valleys of South Carnarvonshire and North Merionethshire in North Wales, appear to be the only known areas where the fly is abundantly found.

H. equina occurs in but a few localised areas in North Wales, and chiefly in the secluded and protected valleys of the southern spur of the Snowdonian mountains. On making an investigation of most of these valleys and taking notes of the approximate numbers found, a striking disparity was remarked in the prevalence even in neighbouring valleys. The reason for this limited distribution has been one of the chief objects of the investigation. The fly is distributed as follows:—in the valley which runs from Portmadoc to Beddgelert it occurs, but not very commonly, and also in the smaller lateral valleys. Somewhat further to the West lies the deep winding valley of the river Dwyfawr, known as the Pennant Valley; here the fly is abundant. This valley is chiefly given over to sheep farming, but on the lower lands a number of cattle are kept, and owing to the habit of milking the cows out of doors in summer, the cattle were more readily approachable and the work of the observation thus rendered easier. Most of the field work, therefore, was carried

out at this valley, and chiefly about the little village of Llanfihangel-y-Pennant, which is conveniently situated near the main road leading from Tremadoc to Garn Dolbenmaen, and above which the valley runs for a distance of five miles into the hills. Another smaller valley running almost parallel to the Pennant, and known as Cwm Ystradlyn, has also supplied data. All these valleys lie in the main north-east to south-west direction. The upper reaches are steep ascents merging into mountain, while the lower portions are wooded. The valley bottoms are wide and level and well suited to the typical hill farming of cattle and sheep rearing. There is a mean annual rainfall of 45 to 50 inches.

The village of Beddgelert and district is commonly supposed to be the real habitat of *H. equina*, and the insect is locally known as the Beddgelert Fly, but it has not been found so abundantly there as in the Pennant valley. *H. equina* is also found on the northern slopes of the Snowdonian mountains, in the valley running up from Llanberis, about a small village known as Nant Ucha, an important route for stage coaches in the past, but now almost exclusively used for motor traffic. Many of the older inhabitants speak of the way horses used to be worried and terrified, but its occurrence there is now rare. The fly is also known to occur about Bala and Llanuwchllyn in North Merionethshire.

From an historical point of view it is rather disappointing not to be able to record what is felt would have been interesting reading, dealing with the molestation of coach horses of which frequent mention was heard ; but the information gleaned seems unreliable, and the passing of time has in no way helped to enhance its value. Suffice it to say that the replacing of horse by motor traffic has caused the fly to become of less economic importance and the old coach roads are particularly free from the pest, which is now concentrated about the cattle lands.

Factors Controlling Occurrence

A succession of wet summers and the distance of the valleys named from Bangor has added considerably to the task of observing the natural habits of *H. equina*. The nights are invariably cold, with heavy dews. When the sun shines the flies are to be abundantly obtained from both horses and cattle, but during cold or rainy days

very few are to be noticed. The entrance to the Pennant Valley is well wooded and cattle grazing in these parts are not so much infested as those higher up the valley, where there are fewer trees. The trees are mostly Ash and Oak, giving way to a dense covering of Bracken (*Pteris aquilina*) which dominates the western side up to where the valley ends ; this side is also steeper than the eastern, which has a more gradual rise and better pasture. Animals grazing on the western side were found to harbour a greater number of flies than those of the opposite side.

This relative prevalence of *H. equina* in one localised area was most fortunate, greatly facilitating the work of comparison and analysis of factors which were supposed to be conducive and favourable to its existence. The geological formation of the area covered is chiefly Cambrian, with its associated shales and slates, this same rock formation being dominant throughout the range. The presence of trees seems in no way necessary to the activities of the flies, and the number obtained was smaller when cattle were sheltering in their cool shade ; there was, also, not much difference in the facies of either side of the valley in their lower and wooded regions. Climatic conditions claimed greater attention, it being well known that *H. equina* is more active during sunshine than at other periods. The western side receives the sun's rays earlier, and thus has a greater period of warmth daily than the opposite slopes which remain cold up to mid-day—this factor may well be of importance. But the most striking difference lies in the abundance of bracken on the western and its comparative scarcity on the eastern slopes. Several farmers had previously suggested an association between *H. equina* and bracken, and acting upon this information a close observation of the habits of the fly has revealed an association which is the main factor for localisation, namely a dependence upon the presence of bracken. During the day *H. equina* are only occasionally found settling on the fronds sunning themselves, but at sunset, or when a spell of cold weather or rain is imminent, they generally leave the cattle and settle on the undersides of the fronds, such a position offering shelter and protection during the night. The main association, however, appears to be during the period of pupation, which will be discussed later.

Hosts

The chief hosts of *H. equina* are horses and cattle, though in their absence the flies are stated to attack other domestic animals, or even man (Neveu-Lemaire, 1912). They usually occupy a position safe from disturbance by the host, generally clustering together under the tail of cows, along the perinaeum, and occurring even as low as between the thighs and on the udder. The skin at these places is thinner than at other parts and without a dense hairy covering, but, the inaccessibility of the parts chosen demonstrate the necessity for freedom from molestation during long spells of feeding. When disturbed, *H. equina* scatter in all directions and exhibit their marked capacity of varied and rapid movements. Many try to conceal themselves in the positions indicated, and only resort to flight as a final alternative. Animals which have been reared in these valleys are so accustomed to the presence of the fly that little or no resentment is shown at their presence. In the country investigated *H. equina* has only been observed on cattle and horses ; but a very interesting report has been received from one of the chief sheep owners who states that he has observed the pest on dogs. Young sheep dogs (not having completed their training) are stated to have been attacked in each of three cases noted ; the older dogs are described as running through the bracken with their heads held high, whilst these young dogs, through keeping their heads too near the ground were attacked. The infested dogs, holding their heads to one side, make efforts to rid themselves of the pest with their paws, the insects being eventually found on the inner surface of the pinna of the ear. This evidence is borne out by the statements of both Neveu-Lemaire and Buxton (*op. cit.*) in their records of the observation of *H. equina* on dogs. A closely related species *H. capensis*, v. Olf. (*H. canina*, Rond), is always found in large numbers on the head and neck of pariah dogs in the near East (see Buxton, *op. cit.*). Unfortunately the writer is unable to confirm these statements from personal observation.

In most of the districts, cattle are sent out to pasture in early summer, and are not brought indoors again until the cold weather sets in. The majority of the cattle kept in the hilly districts are Welsh Blacks or crosses of that breed, and tables of occurrence of the

pest were kept to ascertain whether the lighter coloured animals were more liable to infestation. Little difference was found in the aggregate, and the following data from a typical farm show the colour of cattle, and the number of *H. equina* found. This indicated that both light and dark coloured cattle are similarly infested. These figures were obtained on August 2nd, six cattle yielding the following : Black, 17 flies ; Black, 9 ; Black, 4 ; Blue Grey, 15 ; Roan, 10 ; Blue Grey, 4 ; giving a rather low average of 9·8.

Mode of Dispersal

Residents of the infested areas state that *H. equina* does not migrate of its own accord, but is distributed by host animals, and all evidence points to this view being correct. The mountain ranges are almost impassable barriers to insects that seldom fly more than a few yards, and unless strong winds carry them (which is very improbable) they are entirely dependent for their distribution on the movement of cattle and horses. When cattle are driven or taken away from their pasture, some of the flies present upon them may adhere and accompany them for considerable distances. This is well known to the local farmers. One of the chief reasons for the failure of the fly to extend its range, appears to be the fact that such flies are practically invariably caught and destroyed. The presence of one fly is sufficient to terrify animals not inured to it, the cattle racing wildly to be rid of it. Stallions from the infested areas, travelling the countryside, have been known to introduce the fly to new districts, but owing to the scrupulous care taken in destroying the pest, and the unsuitability of new environment, they soon die off. The following instance was brought to the notice of the writer. A farm outside the infested area was visited by a stallion from the Pennant Valley, carrying with it some of the flies. This led to trouble, mares becoming excited and restless, until every fly had been destroyed. Another instance of the annoyance caused by their presence was given by a blacksmith who had been shoeing a horse from an infested area, and which had left this some time previously. When a horse from a non-infested area was brought in, a fly that had left its host and was present in the smithy, rendered the shoeing impossible until the insect was detected and destroyed.

COLLECTING, ETC.

H. equina is easily caught by hand, there being little risk of damaging the flies by this method owing to the tough and leathery consistence of the integument ; another method is to place a wide-mouthed bottle over a cluster. A forceps net eventually proved most successful for collecting them in numbers. Great difficulty was experienced in attempting to keep the fly in the live state for laboratory experiments, the failure of which necessitated relying entirely upon field observation. When the flies were transferred direct to breeding cages, they died within 48 hours, a few only surviving that period. All the gravid females (distinguished by their swollen abdomens) were placed in separate breeding cages, but clustering together deposited their larvae prematurely. When a single gravid female is placed in a tube there is the same premature deposition, within less than an hour in many cases. A final trial was made by arranging a layer of peat covered with young bracken, and transferring gravid females direct from the host into the cage containing it. The deposition in this instance was delayed and the larvae obtained turned black in colour but no imagines were bred out. Massonnat and Vinet (1913) also complain of the great difficulty that attended their efforts to produce adults.

These unsuccessful attempts at rearing left but one course open for the observation of the deposition of larvae and their development—a prolonged investigation in the field during periods of suitable weather. Well advanced gravid females were caught and a little cotton wool fixed to the underside of the abdomen with a drop of gum. This enabled a close watch to be kept on the activities of those marked, and to follow their movements when they left the cattle. Here may be noted the great advantage obtained in having one or more cows which will not heed the presence of the observer. After marking a few females in the morning it was found necessary to remain with the cattle for the greater part of the day.

HABITS

For the greater part of the day *H. equina* rarely leave the host animal, but during cold or wet weather they are often found on the undersides of the bracken fronds. Occasionally they have been noticed sunning themselves either on the bracken fronds or on the

slabs which abound in the valley, but their activities are chiefly confined to blood sucking. Ormerod (1900) states that *H. equina* feeds on 'the perspiration given off by cattle during the period of their activity in the summer months' besides blood sucking. The nature of the mouth parts, with their narrow piercing stylet curving downwards and forwards and terminated by a distinct cutting apparatus, would seem to leave no doubts as to the nature of the chief food supply. The length of the proboscis is of some importance, that of *H. equina* being about the longest met with during an examination of the mouth parts of other Hippoboscidae; this is, no doubt, a necessity for the successful penetration of the hides of horses and cattle. It is still a matter of opinion whether the flexibility of the proboscis and its sweeping of surfaces allow the admissibility of Miss Ormerod's assumption, and moreover it is a very difficult matter to prove. In the writer's opinion the act of sweeping the surface is thought to be for the locating of a suitable spot for puncturing; and the length is an essential adaptation for reaching the blood-vessels.

Flight

The wings of *H. equina* are well developed, and it is a strong flier, but rarely makes flights of longer duration than is necessary to reach the bracken.

Numbers

The number of flies seen on any one animal varies considerably; as many as thirty may be obtained in some cases, though generally they range from ten to twenty. They are to be found in their greatest numbers on the part immediately below the genitalia and are only occasionally met with in the inguinal region, on the udder and the perinaeum.

The first appearance of *H. equina* is variable, being dependent to a great extent on weather conditions; they have been known to appear as early as April, but their usual time is May. The height of infestation is towards the middle of August and early September, when new individuals are appearing; there is then a sharp falling-off in numbers during the latter end of September, although a few persist into October.

Proportion of Sexes and Copulation

There is no great disparity in the proportion of sexes, the females being in a very slight majority. Copulation has always been observed to take place on the host animals; the male, without any preliminaries, grasps the female and remains in this position for but a short time.

Breeding Habits

The gravid females are readily distinguishable by their distended abdomen. When the larva is mature the females leave the host, but it is a matter of great difficulty to observe the act of deposition, which takes place among the organic débris that collects at the base of the stems of bracken (*Pteris aquilina*). After leaving the host the females settle on a frond of bracken and, dropping to earth, choose a situation in the decaying humus where the larva is deposited. The writer has observed this on five separate occasions all during early August. The larva is partially buried in the humus as soon as extruded. It is of a globular shape and creamy white in colour at extrusion, with a black cap and two conical projections at that pole. It is incapable of any individual movement and has little or no trace of segmentation. It pupates after the passing of a few hours, the larval integument simply becoming chitinised to form the pupal casing, whilst a gradual darkening of the integument takes place until the puparium is black. It is not essential for the larva to be placed in suitable surroundings for pupation as this will take place, in many instances, in a glass tube or other receptacle. In addition to the five cases of actual larval deposition in decaying humus noted above, the writer discovered twelve pupae among organic débris beneath the bracken. Further, two pupae have been discovered on pasture land (not far from bracken) lying in crevices of twisted roots of grass. It is believed that this is an unusual occurrence. The nature of the decaying humus beneath the bracken lends support to the assumption that this is the normal habitat of the pupae, as nearly all observed have been found here, and the actual deposition of larvae has been observed to occur here. In this position there is shelter from heavy rains, while moisture drips down from the fronds. The sun does not penetrate strongly.

and a continuous moisture is assured. The decay of the humus possibly supplies a certain amount of warmth during decomposition.

Climatic conditions may play an important part in determining the duration of the pupal instar, for during periods of hot weather newly hatched individuals were noted on the cattle. This is possibly due to a shortening in the duration of the instar owing to the warmth. The numbers are always greatest on cattle during such times.

In no case has the writer succeeded in hatching out any imagines under artificial conditions, despite a number of attempts, using breeding cages and varying temperatures and conditions.

SUMMARY

1. *Hippobosca equina* has a limited distribution in North Wales, and is restricted to certain valleys in South Carnarvonshire and Merionethshire. Its distribution seems to be governed by two factors :—(1) Presence of bracken, on which depends successful pupation, (2) The amount of sunshine available.

2. The chief hosts of *H. equina* are horses and cattle, although evidence is available to show that it attacks dogs.

3. Extension of range seems to be kept in check by a policy of destruction when stock are removed from its haunts or when an individual is noted in a new district.

4. As an economic pest it is a source of annoyance in terrifying animals not accustomed to its presence, and may then give rise to grave consequences. This factor appears of less importance since the diminution of horse transport. It now occurs almost entirely on cattle and confines its activities to blood sucking.

5. It was found impossible to keep the fly alive and conduct breeding experiments under laboratory conditions.

6. The fly is generally found from May to August.

7. Copulation takes place on the host animals.

8. From observation it seems probable that the decaying humus beneath growing bracken is the normal habitat for deposition of larvae.

ACKNOWLEDGMENTS

It gives the writer much pleasure to acknowledge his indebtedness to Dr. C. L. Walton, Adviser in Agricultural Zoology, University College of North Wales, Bangor, at whose suggestion these observations were carried out, for most willing and helpful guidance and advice. Also, to Professor P. J. White, M.B., F.R.S.E., Department of Zoology, for his stimulating interest and suggestions during the conduct of the work.

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REPORT ON THE INVESTIGATION INTO THE DESTRUCTION OF VERMIN BY HYDROGEN CYANIDE, WITH ESPECIAL REFERENCE TO BED BUGS

BY

R. NEWSTEAD, F.R.S.

ALWEN M. EVANS, M.Sc.

AND

W. H. POTTS, B.A.

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PLATE II

This investigation was carried out at the request of the Liverpool Port Sanitary Authority.

The work was done with the collaboration of Professor W. H. Roberts, Drs. W. Hanna and F. C. White.

The object of the enquiry was to determine the efficacy of various strengths of Hydrogen Cyanide in the destruction of vermin, especially bed bugs, under natural conditions on board ship.

Preliminary experiments with this gas were conducted in a lethal chamber on the roof of the Public Health Laboratory, the final experiments on board ship.

A summary of the experiments is given on p. 117, and recommendations as to the use of Hydrogen Cyanide on p. 118.

HAUNTS OF THE BED BUG

Certain bug-infested houses and quarters on board ship were inspected for the purpose of ascertaining the various conditions under which these insects live. The conditions observed were, as far as possible, reproduced in the test experiments with the hydrogen cyanide.

Bed bugs, as their name suggests, are to be found chiefly in bedrooms and sleeping quarters, places which will afford them an

opportunity of feeding on their host (man) during the night. Being insects which shun light, they withdraw during the day to any retreat which will give them shelter from the light. From these places they come out only at night for the purpose of feeding. The eggs are laid in their day-time haunts.

In houses, the situations favoured by these insects are :—cracks between woodwork fittings and the wall, such as are afforded by brackets or racks nailed or screwed on to the wall ; by badly fitting door frames and mantel-pieces ; behind pictures, especially underneath the paper backing where this is broken ; behind old wall-paper which is peeling off the walls ; cracks in plaster ; hangings, such as curtains, or mantel-covers ; bed-frames, especially in the case of bedsteads with hollow or tubular iron frames.

In ships similar conditions will afford shelter to the bugs, but one or two special ones require attention. Thus the tongue and groove boarding, which so often covers the partitions, or two thicknesses of which form the actual partition, forms a very good refuge, especially when there is a certain amount of air space behind the tongue and groove boarding, into which the bugs can penetrate.

The frame-work of the bunks also seems to be of some importance. In one ship that was investigated, the bunks were put up in sections, and the joints were furnished with collars with slots (see Plate II, fig. 1), in which accumulations of cast skins and living bugs were found. Of greater importance, however, was the fact that the frames of certain types of bunks were hollow tubes with small openings at the ends (fig. 3, B). In the case of the upright stanchions (fig. 3, C), the top end fitted loosely into a socket, whilst the bottom end was let into the deck. The loose fitting socket at the top was of such a nature as to allow the bugs easy access into the tube, whilst the gas, owing to its lightness, would penetrate down the tube only slowly and with difficulty.

A third form of refuge on board was found in a pile of life-jackets observed on one ship. In the folds of the canvas covering of these, bugs were found, and it was thought possible that the insects might penetrate to their interior. Piles of bedding, old clothes, and other such articles might form a similar refuge.

Most of the situations which have been mentioned—crevices in wood-work, cracks in plaster, etc., do not afford the bugs efficient

protection against the gas. Three cases, however, required special attention :— (1) match-boarding with a cavity behind, into which the bugs could retreat ; (2) tubular iron bunk frames ; (3) life-jackets, piles of bedding, old clothes, etc. The first of these cases was investigated by means of a specially constructed box which will be described below ; the second, by means of glass tubes, as will also be described below ; and the third, by using similar life-belts.

DESCRIPTION OF APPARATUS USED

Pill Boxes (card-board). Those used were about 5 cms. in diameter, and 3·5 cms. in height. They proved to be readily permeable to the Hydrogen Cyanide, and appeared to afford no protection to the bugs.

In the first experiments, in order that some sort of protection from the gas should be afforded, the bugs were placed between two layers of felt in the bottom of the pill box, which was then loosely packed with cotton wool, flannel, paper, etc. This packing appeared to make no difference to the efficacy of the gas.

Glass Jars (fig. 1). In many of the experiments the pill boxes were placed inside glass jars of the type used for preserving fruit. These jars had a capacity varying between 930 and 960 c.cs., having

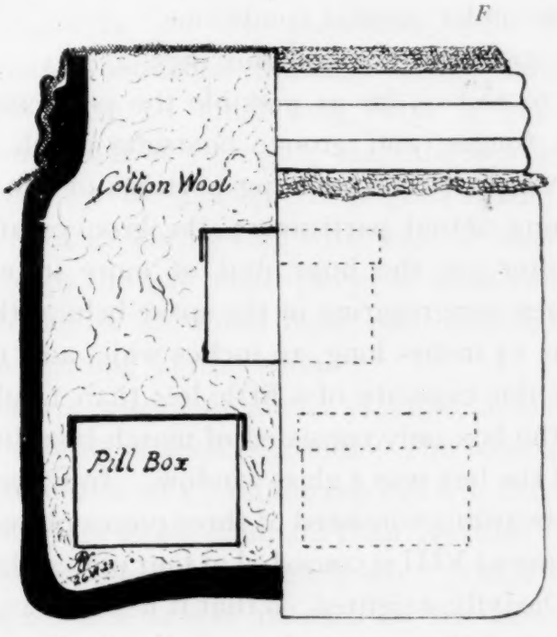

FIG. 1. Section and elevation of the glass jar used in the experiments. *F.*—Flannel covering.

a height of about 12 cms. and a diameter of about 10 cms. In ordinary use the grooved metal ring clamps down a flat metal disc over the top of the jar, thus hermetically sealing it ; for the purpose of the experiment, the flat metal disc was replaced by a piece of flannel readily permeable to the gas.

The jars were used in two ways :—

(1) The pill boxes containing the bugs were placed on the bottom of an empty jar, the mouth of which was closed with flannel as described above.

(2) The pill boxes were placed in the middle of cotton wool which filled the jar, as is shown in fig. 1.

Lethal Chamber. In the Experiments I-VIII, the exposure to the Hydrogen Cyanide was effected by placing the various pieces of apparatus containing the bugs in a strong wooden chest, referred to as the ' Lethal Chamber.' For a description of this and details of the strength of gas used and method of generation see the Chemist's report (p. 118).

The apparatus so far described, was designed for a preliminary test of the efficacy of the gas, and the protection afforded to the bugs in no way closely imitated the protection available to them in their natural haunts on board ship. The pieces of apparatus to be described below were designed especially to imitate certain of the refuges available under natural conditions.

The Tongue and Groove Board Box (figs. 2, 2, A). This box was made in order to test as far as possible the protection afforded to insects by the tongue and groove boarding which is used more particularly in ships, either covering portions of the ' skin ' of the vessel, or forming actual partitions ; the grooves afford a certain amount of shelter for the bugs, but of more importance is the possibility of their congregating in the space behind the boarding.

The box was 14 inches long, 10 inches wide, and 12 inches high, having thus a cubic capacity of a little less than 1 cubic foot. One of the sides of the box only consisted of match-boarding, and on the opposite side of the box was a glass window. In Experiments V and VII the match-boarding consisted of three pieces placed horizontally, whilst in Experiment VIII it consisted of four pieces placed vertically.

The lid was heavily weighted, so that it fitted down closely on the top of the box, and the grooves formed practically the only means by which the gas could penetrate to the interior of the box.

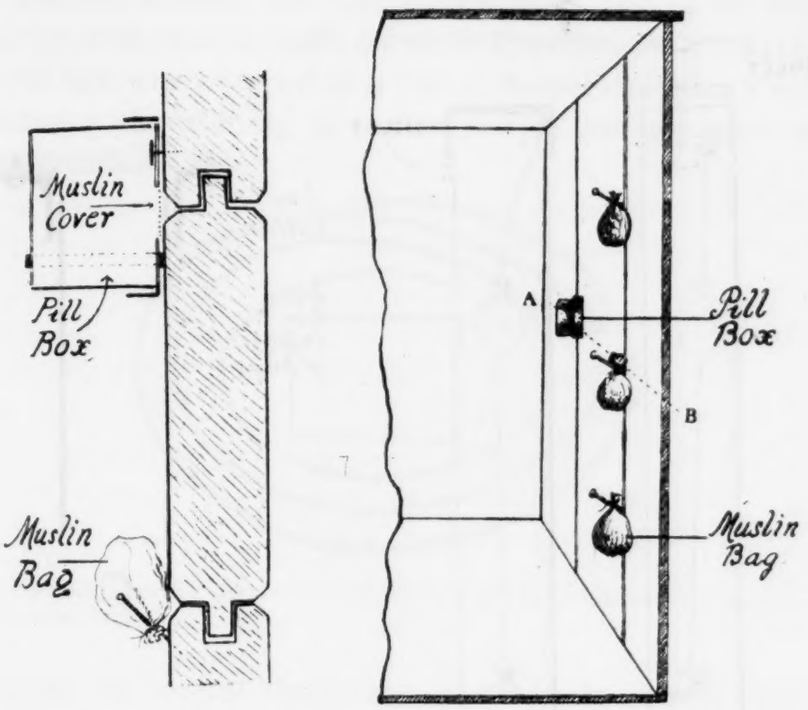

FIG. 2. Tongue and groove board box: section of part of the interior showing the relative positions of the pill box and muslin bags containing bugs. 2A.—section of the tongue and groove boarding (scale ½), showing the positions of the pill box and muslin bag in relation to the joints of the timber.

The Glass Tubes (fig. 3). Two glass tubes, I and II, were arranged so as to imitate the conditions found in certain bunks (see fig. 3, B). Tube I was roughly 48 cms. long, and 4 cms. in diameter, having a volume of about 530 c.cs.; the tube was closed at each end by corks, pierced by two short pieces of glass tubing about 8 mm. in diameter. The corks were sealed with wax, so that gas entered the tube only through the two small pieces of glass tubing. This gave the bugs such shelter as would have been afforded by those bunk tubes with an opening at the ends.

Tube II had a length of about 51 cms., a diameter of about 4 cms., and a volume of about 590 c.cs. The bottom end was closed by a cork covered with wax; on the top end was fitted the lid of a pill box, having a slightly larger diameter than the tube, and raised from it by a small piece of plasticine on each side. There was thus a small inlet for the gas, such as was afforded by the loosely-fitting socket of the upright stanchion of the bunk (fig. 3, C).

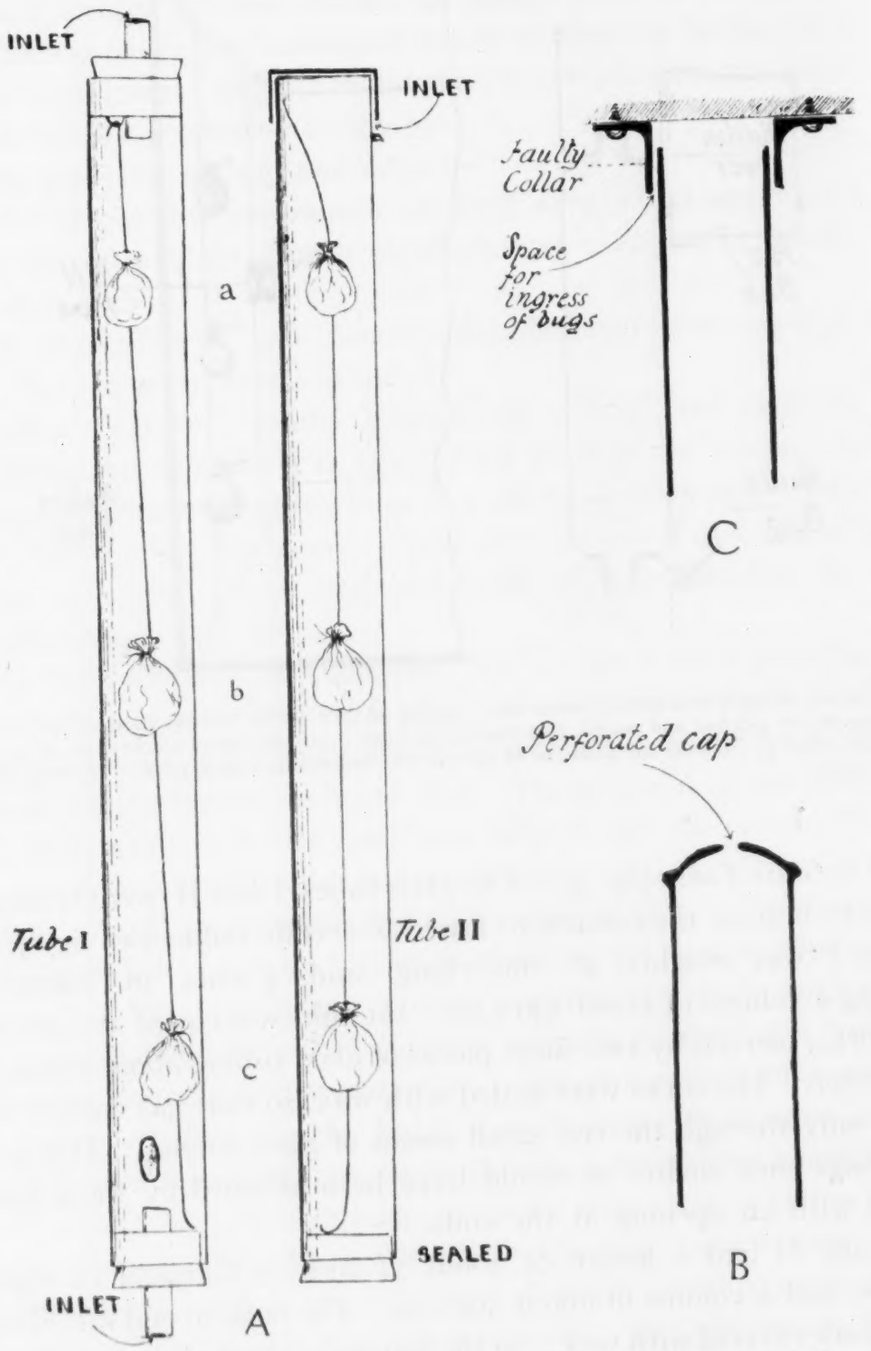

FIG. 3. *A*.—The glass tubes used in the experiments to test the viability of the bugs and the powers of diffusion of the gas under conditions illustrated in *B* and *C*. *a*, *b*, *c*—muslin bags containing bugs. *B*.—Schematic section of the end of a tubular iron bedstead (faulty type), showing the perforated cap through which the bugs gain access to the tube. *C*.—Schematic section of the tubular stanchion with loose-fitting (faulty) collar, leaving space for the ingress of bugs.

Life-Jackets, Bedding, etc. As a preliminary test of the amount of protection such objects might afford, in Experiments Vc, VIIc, and IXb, a pill box was wrapped in a roll of flannel and cotton wool (a cross section is shown in fig. 4) so that the pill box was protected in

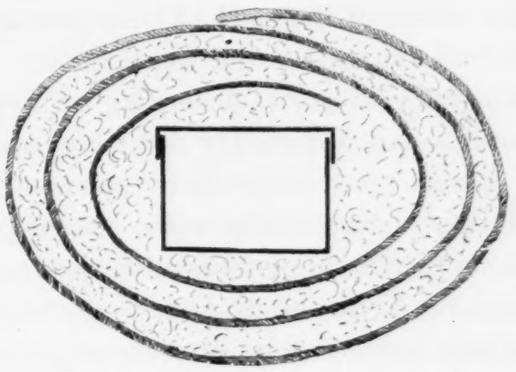

Fig. 4. Schematic section of the roll of cotton wool and flannel showing the position of the pill box containing bugs.

all directions by several thicknesses of alternate cotton wool and flannel. This protection being proved to be of no value to the bugs, sterner tests were carried out.

In Experiment VIIf, a life-jacket (Plate II, fig. 2), 2 ft. 8½ in. long, 10½ in. wide, and 3 in. in height was used. This life-jacket was stuffed with tightly-packed Kapok (a vegetable product resembling raw cotton). The pill box was placed inside the middle portion (Plate II, fig. 2x), and the end portion tied firmly over the middle portion, the resultant being a compact roll, 10 in. by 10½ in. Even this failed to prevent a fatal concentration of gas reaching the bugs.

In Experiment IXk, a third test was carried out, the pill box being inserted in the interior of a straw-stuffed mattress. This, too, failed to protect the bugs against the gas.

LABORATORY METHODS OF DEALING WITH BUGS

In the Laboratory the bugs were kept between layers of dark-green baize in glass jars 3½ in. by 2 in., or tubes 3 in. by 1 in., covered with a layer of cotton voile, and the jars and tubes placed in an incubator at a mean temperature of 25° C. At intervals of about a week the bugs were given an opportunity of feeding on the shaved abdomen of a rabbit. The voile-covered jars were applied to the

host's skin, and the bugs had no difficulty in feeding to repletion in this way. We are indebted to Dr. J. W. Scott Macfie for conducting these operations. In addition many batches of larval bugs were fed on the investigators.

Great care had to be taken in deciding whether bugs which had been exposed to the action of the gas had been permanently affected or not. It was found that after the experiments, bugs could be divided roughly into four categories according to their condition :—

(1) Bugs motionless and apparently dead immediately after the experiment, and never recovering their powers of movement.

(2) Bugs motionless immediately after the experiment, after the lapse of 24 hours regaining imperfectly their powers of movement, but after this becoming feebler daily and finally dying.

(3) Bugs motionless immediately after the experiment, but after about 24 hours completely regaining their powers of locomotion, after which they continue to live in a normal way.

(4) Bugs which are quite active and apparently healthy immediately after the experiment and remain so.

There was no doubt that bugs belonging to categories 1 and 2 were killed by the gas, but it was less easy to decide whether those in 3 and 4 had not suffered some permanent injury which would result in their ultimate destruction. The surviving bugs of categories 3 and 4 were, therefore, kept under observation for several days and tested to see whether they were able to feed, and produce fertile eggs, before they were considered to have recovered completely from the effects of the gas. Several batches of eggs obtained from the survivors were allowed to hatch and the larvae which emerged were quite healthy and fed readily when placed upon a host. It was abundantly evident from these observations that the bugs were in no way injured by the gas and would have been quite capable of continuing the infestation of either houses or ships.

At the beginning of the investigation controls were used for both adults and eggs of *C. lectularius*. These were put into receptacles similar to those containing the experimental specimens and taken to the place where the experiment was conducted, remaining near the lethal chamber till the close of the experiment. This was done in order to test the effect of the sudden lowering of temperature upon the bugs. As the control bugs remained entirely unaffected, we

considered it unnecessary to use them for the adults and nymphs in later experiments, the stock lot of bugs serving for comparison with the survivors during the period of observation. In the case of eggs, however, control eggs laid on the same day were kept under observation to insure that there was no defect inherent in the eggs to prevent their hatching.

EXPERIMENT I. 7.6.23.

Material used in this experiment.

Bed bugs (*Cimex lectularius*).

A and *C*. 12 bugs in each. *Controls:* for *A* and *C*. 12 bugs.
B and *D*. 12 eggs in each. *Controls:* for *B* and *D*. 13 eggs.

Conditions.

General. The material was exposed for two hours to a concentration of Hydrogen Cyanide of about 0·3 per cent.

Details. *A* and *B*. The specimens were placed between two layers of green felt in chip-boxes, and the latter filled loosely with flannel. The boxes were placed in a glass jar, covered with flannel.

C and *D*. The specimens were placed between two layers of green felt in chip boxes and the latter packed with cotton wool. The boxes were placed in a glass jar which was also packed with cotton wool and covered with flannel.

Results.

A, *B*, *C*, and *D*. Both bugs and eggs were all killed in each case.

Controls. Bugs. All were alive on the next day and had laid seven eggs during the night after the experiment. Eggs. 10 out of 13 (77 per cent.) hatched between 10th and 12th June.

EXPERIMENT II. 8.6.23.

Material used in this experiment.

Bed bugs (*Cimex lectularius*).

A. 12 eggs. *Controls:* 12 eggs (laid same day).
B. 6 bugs. *Controls:* 6 bugs.
C. 9 eggs. *Controls:* 9 eggs (laid same day).

Conditions.

General. The material was exposed for one hour to a concentration of Hydrogen Cyanide of about 0·3 per cent.

Details. *A*. The specimens were placed between layers of green felt in a chip box, and the latter loosely filled with flannel, and placed in an empty jar.

B and *C*. The specimens were placed between layers of green felt in a chip box, the latter packed with cotton wool, and placed in a jar also packed with cotton wool.

Results.

A. All the eggs were killed.
Controls. All hatched.

B. Of the 6 bugs, 3 survived.
Controls. All were quite normal on the morning after experiment.

C. All the eggs were killed.
Controls. 5 out of 9 (55 per cent.) hatched.

EXPERIMENT III.

Head louse (*Pediculus capitis*).
A. Adults and eggs. *Controls:* eggs.

Body louse (*Pediculus corporis*).
B. Adults.

Conditions.

General. The material was exposed for two hours to a concentration of Hydrogen Cyanide of about 0·3 per cent.

Details. The specimens were placed in chip boxes in petri-dishes and the dishes filled up with tightly-packed cotton wool and covered with one layer of flannel.

Results.

The eggs and adults were all killed by the experiments.
Controls. The eggs hatched normally.

EXPERIMENT IV. 13.6.23.

Material used in this experiment.

Bed bugs (*Cimex lectularis*).

A. 30 bugs. *Controls:* 6 bugs.
B. 6 bugs. *Controls:* 4 bugs.
C. 45 eggs. *Controls:* 12 eggs.
D. 11 eggs. *Controls:* 10 eggs.

Head lice (*Pediculus capitis*).

E. Eggs. *Controls:* eggs.

Body lice (*Pediculus corporis*).

F. Adults. *Controls:* adults.

Conditions.

General. The material was exposed for three hours to a concentration of Hydrogen Cynaide of about 0·2 per cent.

Details. A and *C*. The specimens were placed between two layers of baize in chip boxes and the boxes filled up tightly with cotton wool; the boxes were placed in jars and the jars packed with cotton wool and covered with a layer of flannel (see fig. 1).

B and *D*. The specimens were placed between two layers of baize in chip boxes and the boxes filled up with flannel and then placed in otherwise empty jars covered with flannel.

E. The eggs attached to the hairs on which they had been laid, were placed in glass-bottomed pasteboard boxes with tight-fitting pasteboard lids.

F. The lice were among the folds of a garment which was rolled up and placed in a glass jar covered with flannel.

Results.

A. 10 out of 30 bugs were killed.
B. 2 out of 6 bugs were killed.
C. 12 out of 45 eggs hatched (i.e., 27 per cent.).
Controls: 8 out of 12 hatched (i.e., 67 per cent.).
D. 6 out of 11 eggs hatched (i.e., 54 per cent.).
Controls: 7 out of 10 hatched (i.e., 70 per cent.).
E. All the eggs hatched.
F. None of the adults was killed.

EXPERIMENT V. 19.6.23.

Material used in this experiment.

Bed bugs (*Cimex lectularius*).

A. 20 bugs. *Controls:* 12 bugs.

B. 48 eggs. *Controls:* 29 eggs.

C. 20 bugs. *Controls:* those used for *A*.

D. 20 bugs. *Controls:* those used for *A*.

Black Rats.

E. 3 living rats from ship.

Conditions.

General. The material was exposed for three hours to a concentration of Hydrogen Cyanide of about 0·2 per cent.

Details. A and *B*. The specimens were placed between folds of green baize, in a chip box with two holes in the lid, over which had been pasted voile. The box was then pinned inside the match-boarding box (fig. 2, A), so that the two holes in the lid of the former were lying opposite the groove between the match-boarding of the latter.

C. The specimens were placed between folds of baize in a chip box, and the latter wrapped up in cotton wool and flannel (see fig. 4), forming a roll of about 4 inches in diameter, in the centre of which was the chip box.

D. The specimens were placed in a chip box between folds of baize. The lid of the chip box was perforated with small holes, and the chip box placed on the floor of the lethal chamber.

E. The rats were placed in a small cage on the floor of the lethal chamber.

Results.

A. Bugs. The whole number (20) recovered by the next morning.

B. Eggs. 37 out of the 48 (77 per cent.) hatched.

Controls. Of the 29 eggs, 28 hatched.

C. Bugs. 19 of the 20 completely recovered by the next morning.

D. Bugs. All were killed.

A, *C*, and *D*. *Controls*. Bugs. All 12 quite normal on the next morning.

E. The three rats were stiff when taken out of the lethal chamber. From them were collected the following :—9 ♂ ♂ and 4 ♀ ♀ of the plague flea (*Xenopsylla cheopis*), all of which were dead.

EXPERIMENT VI. 22.6.23.

Material used in this experiment.

A. 15 rats from a warehouse ' black rats.'

B. One rat's nest.

C. 10 larvae of the rat flea (*Ceratophyllus fasciatus*).

Conditions.

General. The material was placed in the lethal chamber for three hours and the concentration used was about 0·2 per cent. of Hydrogen Cyanide.

Details. A. The 15 rats were placed in lethal chamber in a stout unbleached calico bag.

B. The rat's nest was wrapped in paper, which was pierced with slits.

C. 10 larvae of *Ceratophyllus fasciatus* were placed in a tube, the mouth of which was closed by cotton wool, in the rat's nest.

Results.

A. The 15 rats were quite dead, and from them were collected :— 3 ♂ ♂ and 5 ♀ ♀ of *Ceratophyllus fasciatus*, also dead.

B. From the rat's nest we obtained :—One adult *Ceratophyllus fasciatus*, dead, and two larvae, dead.

C Of the 10 larvae in the tube, all were dead when examined on the 22nd and 23rd of June, whilst control larvae were still alive.

EXPERIMENT VII. 25.6.23.

Material used in this experiment.

Bed bugs (*Cimex lectularius*).

A. 20 bugs.

B. 50 eggs. *Controls:* 60 eggs (laid same day).

C. 20 bugs.

D. 10 eggs.

E. Tube I. (*a*), (*b*) and (*c*), 10 bugs. (b^1), 25 eggs.
Tube II. (*a*), (*b*) and (*c*), 10 bugs. (b^1), 25 eggs.

Control: for *D* and *E*, 23 eggs.

F. 20 bugs.

Conditions.

General. The material was exposed for two hours to a concentration of Hydrogen Cyanide of about 0·3 per cent.

Details. A and *B.* The specimens were placed between layers of green baize in a chip box, which was put inside the tongue and groove board chest as in Experiment Va.

C and *D.* As in Experiment Vc.

E. The specimens were placed in muslin bags, (*a*), (*b*), (*b*¹) and (*c*), and these suspended, (*a*) at the top, (*b*) and (*b*¹) in the middle, and (*c*) at the bottom, of two glass tubes, I and II (see fig. 3, A and description of apparatus, p. 95).

F. The specimens were placed in a chip box in the usual manner, and the chip box placed inside a life-belt (see Plate II, fig. 2, and description of apparatus, p. 97).

Results.

A. Bugs. 14 out of 20 (70 per cent.) survived.

B. Eggs. 42 out of 50 (84 per cent.) hatched.
Controls. 47 out of 60 (78 per cent.) hatched.

C. Bugs. All were killed.

D. Eggs. All were killed. *Controls.* All except 2 hatched.

E. Tube I. Bugs. (*a*), (*b*), and (*c*). All were killed in each case.
Eggs. (*b*¹). All were killed.
Tube II. Bugs. (*a*) All (10 out of 10) were killed.
(*b*) 9 out of 10 were killed.
(*c*) 1 out of 10 was killed.
Eggs. (*b*¹) 17 out of 25 (68 per cent.) hatched.
Control. Eggs. All hatched except 2 (91 per cent.).

F. Bugs. All were killed.

Experiment VIII. 13.6.23.

Material used in this experiment.

Bed bugs (*Cimex lectularius*).
A, *B*, (*a*), (*b*), (*c*); *C* (*a*), (*b*), (*c*). 10 bugs in each.
B (*b*¹) and *C* (*b*¹), 20 eggs in each. *Controls:* 8 eggs (laid on same day).

Conditions.

General. The material was exposed for three hours to a concentration of Hydrogen Cyanide, of about 0·3 per cent.

Details. A. The specimens were placed in an open voile bag in a chip box as used in Experiment VA, and the box placed under the same condition as in that experiment.

B. The specimens were placed in voile bags (*a*), (*b*), (*b*¹) and (*c*), in a glass tube (see fig. 3, A), (*a*) and (*c*) being at each end and (*b*) and (*b*¹) in the middle. The tube was supported horizontally in the lethal chamber in such a position that bag (*a*) was nearest to the point of evolution of the gas.

C. The specimens were placed in voile bags (*a*), (*b*), (*b*¹) and (*c*), which were pinned against the groove on the inside of the match-boarding box (see fig. 2), (*a*) being at the top, (*b*) and (*b*¹) in the middle and (*c*) at the bottom.

Results.

A. 4 out of the 10 bugs were killed.

B. (*a*), (*b*), (*c*). All were killed.

B. (*b*¹). None of the eggs hatched. *Controls:* all hatched.

C. (*a*) and (*b*). 5 out of 10 bugs were killed in each case.

C. (*c*). 9 out of 10 bugs were killed.

C. (*b*¹). 12 out of 20 eggs hatched. *Controls:* all hatched.

EXPERIMENTS ON BOARD SHIP

EXPERIMENT IX. 20.7.23. *Fumigation of the s.s. 'Lady Emerald.'*

Material used in this experiment.

A-K. Ten voile bags, each containing 10 bugs, were used.

L and *M.* Two cages of rats, containing 4 and 3 respectively.

Conditions.

General. The material was exposed for two hours to a concentration of Hydrogen Cyanide produced by 8 oz. of Sodium Cyanide per 1,000 cubic feet (i.e., about 0·3 per cent. Hydrogen Cyanide). The gas was generated in tubs, in the usual manner, in the two places fumigated (the seamen's and the firemen's quarters); the position of these tubs in relation to the dispositions of the material may be seen quite readily from figs. 5 and 6.

There were no traces of bugs in the ship, but the food-lockers were very much infested with mice.

Details (see figs. 5 and 6). *A-K.* The voile bags containing the bugs were, for convenience, placed in chip boxes, and these were disposed as follows :—

In the Seamen's quarters (see fig. 5).

A, *B* and *C* were placed, in the match-boarding box used in previous experiments, on a bench, raised about two feet off the floor.

D was placed in a roll of cotton wool and flannel (as used in Experiment Vc.) on the floor beneath the bench mentioned above.

I was placed near to it, to serve as a control.

E was placed in the food-locker, about four feet from the ground.

F was placed on the table in the mess-room.

G was placed on a beam just under the roof.

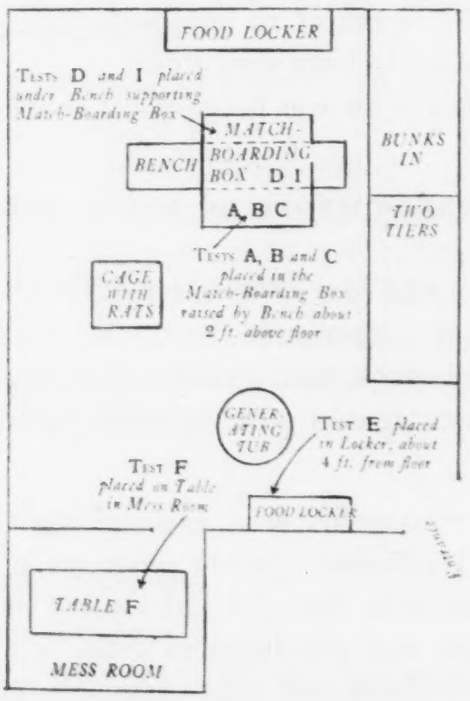

FIG. 5. Schematic plan showing the relative positions of the tests placed in the Seamen's quarters, Experiment IX.

In the Firemen's quarters (see fig. 6).

H was placed in a situation similar to that of G.

K was placed in the straw stuffing of a mattress lying on the bottom bunk on the left-hand side.

L and *M*, the two cages of rats, were placed, one on the floor of the seamen's quarters (see fig. 5), and the other on a bench, raised about 1½ feet above the ground in the firemen's quarters (see fig. 6).

Results.

A. All the bugs survived except one.

B. All the bugs survived except two, though this lot showed a quicker rate of mortality, subsequently, than did either *A* or *C*.

C. All the bugs survived.

D. All the bugs were killed.

E. 3 bugs only out of the 10 survived.

F. 1 bug only (a 3rd stage larva) survived.

G, *H*, *I*, and *K*. All the bugs were killed.

L and *M*. All the rats (7) were killed, and from them were taken 2 specimens of *Ceratophyllus fasciatus* (♀ ♀) and a number of lice, also dead.

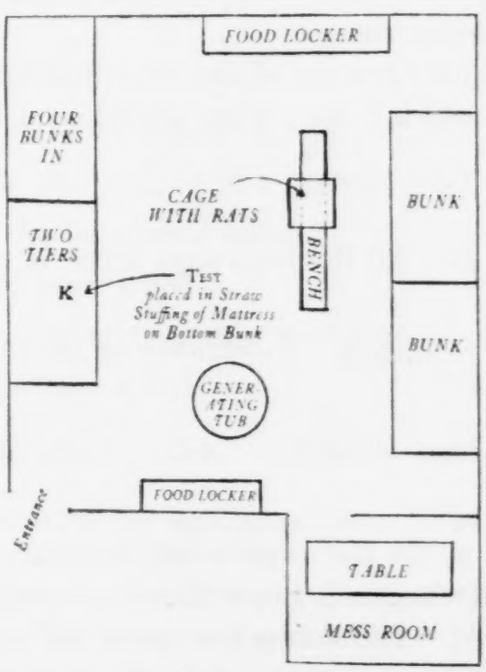

FIG. 6. Schematic plan showing the relative positions of the tests placed in the Firemen's quarters, Experiment IX.

EXPERIMENT X. 23.2.24. *Fumigation of the s.s. 'Montcalm.'*

Material used.

Bed bugs (*Cimex lectularius*).

A, *C* and *D*. 10 bugs.

B. 14 bugs.

Conditions.

General. The passenger accommodation only was fumigated. The fumigation was effected by spraying liquid Hydrogen Cyanide, 2½ oz. per 1,000 cubic feet, giving an average concentration of about 0·27 per cent. HCN. The duration of exposure was from 3-3½ hours.

Details. The bugs were placed in chip boxes loosely packed with pieces of green felt.

A. The chip box was placed behind the skirting-board of a cabin in the stewards' quarters, amidships. The skirting-board was open underneath, and had a couple of large circular holes, the one directly in front of the chip box being closed by means of a pillow.

B. This chip box was used as a control for *A*, being placed on a table in the cabin, to test the concentration of the gas outside the skirting-board.

C. The chip box was placed in a cupboard in a cabin in the passengers' starboard quarters.

D. This was used as a test of the concentration of the gas outside the cupboard in which C was, being placed on a table in the same cabin.

Results.

A, *B*, *C* and *D*. All the bugs were killed in each case.

EXPERIMENT XI. 13.3.24. *Fumigation of the s.s. 'City of Paris.'*

Material used.

Bed bugs (*Cimex lectularis*). *A-G*. 7 lots of 10 bugs.

Conditions.

General. As in the last experiment, fumigation was effected by spraying liquid Hydrogen Cyanide, the concentration produced being about 0·2 per cent. The fumigation lasted for 3-3½ hours.

Details. The bugs were placed in chip boxes with a little green felt and newspaper packing.

A and *B*. The two chip boxes were placed in the rudder post locker, in the firemen's fo'castle. This structure was formed by tongue and groove boarding, about one and a quarter inches thick, with a closely-fitting door, which was closed. The capacity was about 50 cubic feet. The temperature in the fo'castle was fairly high.

C and *D*. These two chip boxes were placed on chests in the firemen's fo'castle, near the rudder post locker. They served as tests of the concentration of gas outside the locker.

E. This chip box was placed in the space under a chest of drawers, in a passenger cabin on the port side of the bridge deck. As this structure was built into the side of the cabin, the only way the gas could penetrate into the space was by means of the crack between the bottom drawer and the framework of the chest of drawers.

F. This chip box was placed in the bottom drawer of the above-mentioned chest of drawers.

G. This chip box was placed on the shelf of a toilet apparatus in the same cabin as *C*. It formed a test of the concentration of the gas in the air outside the chest of drawers.

Results.

A. 1 bug only survived out of 10.

B. 3 out of the 10 survived in a healthy condition.

C and *D*. All the bugs were killed.

E. All the bugs were killed.

F. 2 bugs survived in a healthy condition.

G. All the bugs were killed.

DISCUSSION OF RESULTS AND TABLES

In all the experiments, the action of the gas was not considered to be satisfactory unless every bug was killed. This attitude was adopted because one bug, if it happened to be a fertilised female, would be quite capable of starting a fresh infection.

TABLE I.

Summary of experiments on Bed Bug (*Cimex lectularius*).

Experiments I-IV.

No. of experiment	Conditions of experiment			Experimental material		Control material	
	Average concentration of gas	Length of exposure	Conditions	Number of specimens	Percentage killed	Number of specimens	Percentage died
	%				%		%
IA ...	0·3	2 hours	In pill boxes in empty glass jar, unprotected	12 bugs	100	...	...
IB ...	0·3	2 hours	In pill boxes in empty glass jar, unprotected	12 eggs	100	13 eggs	3
Ic ...	0·3	2 hours	In pill boxes in glass jar, protected by cotton wool	12 bugs	100	...	...
ID ...	0·3	2 hours	In pill boxes in glass jar, protected by cotton wool	12 eggs	100	13 eggs (as used for IB)	3
IIA ...	0·3	1 hour	In pill boxes in empty glass jar, unprotected	12 eggs	100	12 eggs	0
IIB ...	0·3	1 hour	In pill boxes in glass jar, protected by cotton wool	6 bugs	50	...	...
IIc ...	0·3	1 hour	In pill boxes in glass jar, protected by cotton wool	9 eggs	100	9 eggs	45
IVA ...	0·2	3 hours	In pill boxes in glass jar, protected by cotton wool	30 bugs	33	...	...
IVB ...	0·2	3 hours	In pill boxes in empty jar, unprotected	6 bugs	33	...	...
IVc ...	0·2	3 hours	In pill boxes in glass jar, protected by cotton wool	45 eggs	73	12 eggs	33
IVD ...	0·2	3 hours	In pill boxes in empty jar, unprotected	11 eggs	46	10 eggs	30

Table I summarises the results obtained in the preliminary experiments where the only protection afforded to the bugs was that of the cotton wool in which they were packed. A concentration of 0·3 per cent. of gas, acting for two hours, was sufficient to kill both bugs and eggs, whether protected or not (Experiment I). It did not, in one hour, kill bugs when protected by cotton wool (Experiment II). A concentration of 0·2 per cent. of gas, even though allowed to act for three hours, failed to kill either bugs or eggs whether protected or not (Experiment IV).

In the following table, which for convenience has been divided into three sections, are seen the results obtained in the lethal chamber, when forms of protection resembling more nearly those of their natural conditions were afforded to the bugs.

TABLE II.

Summary of the Experiments on Bed Bugs (*Cimex lectularius*).

Section A. Experiment V

No. of experiment	Conditions of experiments			Experimental material		Control material	
	Average concentration of gas	Length of exposure	Conditions	Number of specimens	Percentage killed	Number of specimens	Percentage died
	%				%		%
VA ...	0·2	3 hours	Pill box pinned inside tongue and groove board box	20 bugs	0	...	...
VB ...	0·2	3 hours	Pill box pinned inside tongue and groove board box	48 eggs	23	29 eggs	3
VC ...	0·2	3 hours	Pill box inside cotton wool and flannel roll	20 bugs	5	...	...
VD ...	0·2	3 hours	Pill box with perforated lid on floor of lethal chamber	20 bugs	100	...	...

From this section it will be seen that a concentration of 0·2 per cent., acting for three hours, failed to kill bugs protected by tongue and groove boarding, or by the flannel and cotton wool roll; it succeeded, however, in killing them completely when they were exposed without any means of protection (Experiment V).

TABLE II—*Continued*

Section B, Experiment VII

No. of experiment	Conditions of Experiments			Experimental material		Control material	
	Average concentration of gas	Length of exposure	Conditions	Number of specimens	Percentage killed	Number of specimens	Percentage died
	%				%		%
VIIA ...	0·3	2 hours	Pill box pinned inside tongue and groove board box	20 bugs	30	...	...
VIIB ...	0·3	2 hours	Pill box pinned inside tongue and groove board box	50 eggs	16	60 eggs	2
VIIC ...	0·3	2 hours	Pill box inside cotton wool and flannel roll	20 bugs	100	...	...
VIID ...	0·3	2 hours	Pill box inside cotton wool and flannel roll	10 eggs	100	23 eggs	9
VIIE, I(*a*)	0·3	2 hours	Voile bag suspended at top of Tube I	10 bugs	100	...	...
VIIE, I(*b*)	0·3	2 hours	Voile bag suspended in middle of Tube I	10 bugs	100	...	...
VIIE, I(*b*¹)	0·3	2 hours	Voile bag suspended in middle of Tube I	25 eggs	100	23 eggs (same as used in VIID)	9
VIIE, I(*c*)	0·3	2 hours	Voile bag suspended at bottom of Tube I	10 bugs	100	...	...
VIIE, II(*a*)	0·3	2 hours	Voile bag suspended at top of Tube II	10 bugs	100	...	...
VIIE, II(*b*)	0·3	2 hours	Voile bag suspended in middle of Tube II	10 bugs	90	...	...
VIIE, II(*b*¹)	0·3	2 hours	Voile bag suspended in middle of Tube II	25 eggs	32	23 eggs (same as used in VIID)	9
VIIE, II(*c*)	0·3	2 hours	Voile bag suspended at bottom of Tube II	10 bugs	10	...	...
VIIF ...	0·3	2 hours	Pill box inside life-belt	20 bugs	100	...	...

This section shows that even 0·3 per cent. of the gas failed to kill bugs behind match-boarding in two hours. This concentration also failed, in that period, to penetrate even to the middle of Tube II

in sufficient quantity to kill the bugs. It did, however, succeed in killing completely all those bugs placed inside the flannel and cotton wool roll, in Tube I (the tube open at both ends), and in the interior of the Life-Jacket (Experiment VII).

TABLE II—*Continued*

Section C, Experiment VIII

No. of experiment	Conditions of experiments			Experimental material		Control material	
	Average concentration of gas	Length of exposure	Conditions	Number of specimens	Percentage killed	Number of specimens	Percentage died
	%				%		%
VIIIA ...	0·3	3 hours	Pill box pinned inside tongue and groove board box	10 bugs	40	...	...
VIIIB (*a*)...	0·3	3 hours	Voile bag at end of Tube II furthest from the gas	10 bugs	100	...	...
VIIIB (*b*)...	0·3	3 hours	Voile bag in middle of Tube II	10 bugs	100	...	...
VIIIB (*bl*)	0·3	3 hours	Voile bag in middle of Tube II	20 eggs	100	8 eggs	0
VIIIB (*c*)	0·3	3 hours	Voile bag at end of Tube II nearest the gas	10 bugs	100	...	...
VIIIc (*a*)	0·3	3 hours	Voile bag pinned at top of groove of tongue and groove board box	10 bugs	50	...	...
VIIIc (*b*)...	0·3	3 hours	Voile bag pinned in middle of groove of tongue and groove board box	10 bugs	50	...	...
VIIIc (*bl*)	0·3	3 hours	Voile bag pinned in middle of groove of tongue and groove board box	20 eggs	40	8 eggs (used in VIIIB (*bl*))	0
VIIIc (*c*)...	0·3	3 hours	Voile bag pinned at bottom of groove of tongue and groove board box	10 bugs	90	...	...

Here it is shown that a period of three hours, even, was not sufficient for 0·3 per cent. of the gas to kill the bugs behind tongue and groove boarding, although it did allow the gas to penetrate into Tube II, when placed horizontally, in sufficient concentration to kill all the bugs in it (Experiment VIII).

TABLE II—*Continued*

Section B, Experiment VII

No. of experiment	Conditions of Experiments			Experimental material		Control material	
	Average concentration of gas	Length of exposure	Conditions	Number of specimens	Percentage killed	Number of specimens	Percentage died
	%				%		%
VIIA ...	0·3	2 hours	Pill box pinned inside tongue and groove board box	20 bugs	30	...	...
VIIB ...	0·3	2 hours	Pill box pinned inside tongue and groove board box	50 eggs	16	60 eggs	2
VIIC ...	0·3	2 hours	Pill box inside cotton wool and flannel roll	20 bugs	100	...	...
VIID ...	0·3	2 hours	Pill box inside cotton wool and flannel roll	10 eggs	100	23 eggs	9
VIIE, I(*a*)	0·3	2 hours	Voile bag suspended at top of Tube I	10 bugs	100	...	...
VIIE, I(*b*)	0·3	2 hours	Voile bag suspended in middle of Tube I	10 bugs	100	...	...
VIIE, I(*b*1)	0·3	2 hours	Voile bag suspended in middle of Tube I	25 eggs	100	23 eggs (same as used in VIID)	9
VIIE, I(*c*)	0·3	2 hours	Voile bag suspended at bottom of Tube I	10 bugs	100	...	...
VIIE, II(*a*)	0·3	2 hours	Voile bag suspended at top of Tube II	10 bugs	100	...	...
VIIE, II(*b*)	0·3	2 hours	Voile bag suspended in middle of Tube II	10 bugs	90	...	...
VIIE, II(*b*1)	0·3	2 hours	Voile bag suspended in middle of Tube II	25 eggs	32	23 eggs (same as used in VIID)	9
VIIE, II(*c*)	0·3	2 hours	Voile bag suspended at bottom of Tube II	10 bugs	10	...	...
VIIF ...	0·3	2 hours	Pill box inside life-belt	20 bugs	100	...	...

This section shows that even 0·3 per cent. of the gas failed to kill bugs behind match-boarding in two hours. This concentration also failed, in that period, to penetrate even to the middle of Tube II

in sufficient quantity to kill the bugs. It did, however, succeed in killing completely all those bugs placed inside the flannel and cotton wool roll, in Tube I (the tube open at both ends), and in the interior of the Life-Jacket (Experiment VII).

TABLE II—*Continued*

Section C, Experiment VIII

No. of experiment	Conditions of experiments			Experimental material		Control material	
	Average concentration of gas	Length of exposure	Conditions	Number of specimens	Percentage killed	Number of specimens	Percentage died
	%				%		%
VIIIA ...	0·3	3 hours	Pill box pinned inside tongue and groove board box	10 bugs	40	...	...
VIIIB (*a*)...	0·3	3 hours	Voile bag at end of Tube II furthest from the gas	10 bugs	100	...	...
VIIIB (*b*)...	0·3	3 hours	Voile bag in middle of Tube II	10 bugs	100	...	...
VIIIB (*bl*)	0·3	3 hours	Voile bag in middle of Tube II	20 eggs	100	8 eggs	0
VIIIB (*c*)	0·3	3 hours	Voile bag at end of Tube II nearest the gas	10 bugs	100	...	...
VIIIc (*a*)	0·3	3 hours	Voile bag pinned at top of groove of tongue and groove board box	10 bugs	50	...	...
VIIIc (*b*)...	0·3	3 hours	Voile bag pinned in middle of groove of tongue and groove board box	10 bugs	50	...	...
VIIIc (*bl*)	0·3	3 hours	Voile bag pinned in middle of groove of tongue and groove board box	20 eggs	40	8 eggs (used in VIIIB (*bl*))	0
VIIIc (*c*)...	0·3	3 hours	Voile bag pinned at bottom of groove of tongue and groove board box	10 bugs	90	...	...

Here it is shown that a period of three hours, even, was not sufficient for 0·3 per cent. of the gas to kill the bugs behind tongue and groove boarding, although it did allow the gas to penetrate into Tube II, when placed horizontally, in sufficient concentration to kill all the bugs in it (Experiment VIII).

An interesting fact, brought out by the experiments summarised in the above two tables, is that the eggs of bugs are not more resistant to the action of hydrogen cyanide than are the other stages.

TABLE III.

Summary of Experiments on Bed Bugs (*Cimex lectularius*).

Experiments IX-XI.

No. of experiment	Conditions of Experiments.			Experimental material	
	Average concentration of gas	Length of exposure	Conditions	Number of specimens	Percentage killed
	%				%
IXA ...	0·3	2 hours	Pill box at top of groove of tongue and groove board box	10 bugs	10
IXB ...	0·3	2 hours	Pill box in middle of groove of tongue and groove board box	10 bugs	20
IXC ...	0·3	2 hours	Pill box at bottom of groove of tongue and groove board box	10 bugs	0
IXD ...	0·3	2 hours	Pill box in roll of cotton wool and flannel	10 bugs	100
IXE ...	0·3	2 hours	Pill box in a food-locker	10 bugs	70
IXF ...	0·3	2 hours	Pill box on a table in small mess-room	10 bugs	90
IXG ...	0·3	2 hours	Pill box on a beam under ceiling	10 bugs	100
IXH ...	0·3	2 hours	Pill box on a beam under ceiling	10 bugs	100
IXI ...	0·3	2 hours	Pill box unprotected, near to IXD	10 bugs	100
IXK ...	0·3	2 hours	Pill box in straw stuffing of mattress	10 bugs	100
XA ...	0·27	3-3½ hours	Pill box behind skirting-board of cabin	10 bugs	100
XB ...	0·27	3-3½ hours	Pill box on table in same cabin as XA	14 bugs	100
XC	0·27	3-3½ hours	Pill box in cupboard in cabin	10 bugs	100
XD ...	0·27	3-3½ hours	Pill box on table in same cabin as XC	10 bugs	100

TABLE III—*continued*

No. of experiment	Conditions of Experiments			Experimental material	
	Average concentration of gas	Lengths of exposure	Conditions	Number of specimens	Percentage killed
	%				%
XIA ...	0·20	3-3½ hours	Pill boxes in locker	10 bugs	90
XIB ...	0·20	3-3½ hours	Pill boxes in locker	10 bugs	70
XIC ...	0·20	3-3½ hours	Pill boxes on chests near locker	10 bugs	100
XID ...	0·20	3-3½ hours	Pill boxes on chests near locker	10 bugs	100
XIE ...	0·20	3-3½ hours	Pill box in space under bottom drawer of chest of drawers	10 bugs	100
XIF ...	0·20	3-3½ hours	Pill box in bottom drawer of chest of drawers	10 bugs	80
XIG ...	0·20	3-3½ hours	Pill box on shelf in same cabin as XIE and XIF	10 bugs	100

The experiments summarised in this table were carried out on various ships ; in the first one, fumigation was effected by the dumping method, and in the second and third ones, by using liquid Cyanide with a spray.

In Experiment IX, the concentration was calculated at about 0·3 per cent. of the gas, and the fumigation lasted about two hours ; this failed, again, to kill the bugs behind the tongue and groove boarding ; it failed, also, to penetrate into a food-locker. This experiment illustrates the disadvantage of the dumping method, in that the concentration of the gas is not distributed uniformly throughout the space to be fumigated ; thus, whilst bugs in various positions, including the interior of a straw-stuffed mattress, were killed, some exposed on a table in a small mess-room just off the main one (see fig. 6, p. 107) were not killed.

In Experiment X, a concentration of about 0·27 per cent., acting for three to three-and-a-half hours, was completely successful, all the bugs being killed; the protection afforded was not very great, the skirting-board being open at the bottom, and the cupboard (*C*) not very air-tight.

Experiment XI shows, again, that a concentration of 0·2 per cent., even when acting for three to three-and-a-half hours, is too low to kill bugs if any kind of protection is afforded (e.g., *A*, *B* and *F*), although it does kill those exposed.

TABLE IV.

Summary of Experiments on Lice, Fleas and Rats.

No. of experiment	Conditions of Experiments			Material	Results
	Average concentration of gas	Lengths of exposure	Conditions		
	%				
IIIA ...	0·3	2 hours	In pill box in petri-dish filled with cotton wool	Head lice and eggs	All were killed. *Control.* Eggs were normal
IIIB ...	0·3	2 hours	In pill box in petri-dish filled with cotton wool	Body lice	All were killed
IVE ...	0·2	3 hours	In glass-bottomed paste-board pill-boxes	Head louse eggs	None were killed
IVF ...	0·2	3 hours	In garment stuffed into glass jar	Body lice	None were killed
VE ...	0·2	3 hours	In an iron cage	3 black rats	All killed—13 dead fleas found
VIA ...	0·2	3 hours	In a stout calico bag	15 black rats	All killed—8 dead fleas found
VIB ...	0·2	3 hours	Rat's nest wrapped up in paper pierced by slits	Fleas and larvae	1 flea and 2 flea larvae found dead
VIC ...	0·2	3 hours	In a small glass tube plugged with cotton wool, inside the rat's nest	10 flea larvae	10 larvae all dead. *Controls* remained alive
IXL ...	0·3	2 hours	In a cage on the floor	4 black rats	All were killed, and 2 fleas and a number of lice were found dead on the rats
IXM ...	0·3	2 hours	In a cage on a bench	3 black rats	

From this table it appears that, in the case of lice, a concentration of 0·3 per cent., for one hour, was sufficient to kill both adults and eggs (Experiment III), whilst a concentration of 0·2 per cent., for three hours, was not sufficient (Experiment IV). It is interesting to note that a few of the lice used in this experiment, on the 13th of June, were still alive on the 20th, having spent the seven days off the human body at ordinary room temperature, without any food. They were all dead on June 21st.

Both fleas (adults and larvae), and rats, are killed by a concentration of 0·2 per cent., for three hours, (Experiments V, VI, and IX).

SUMMARY

1. A concentration of 0·2 per cent. of Hydrogen Cyanide does not, even if allowed to act for as long as three hours, with certainty kill every bug.

2. A concentration of 0·3 per cent. of the gas, acting for only one hour, is not sufficient to kill every bug.

3. A concentration of 0·3 per cent. of the gas, acting for three hours, will kill all the bugs present, except where they can retire behind tongue and groove boarding.

4. Eggs of bugs are not more resistant to Hydrogen Cyanide than are the adults.

5. A concentration of 0·3 per cent. of the gas, acting for one hour, is sufficient to kill lice, both adults and eggs ; but a concentration of 0·2 per cent. of gas, even acting for three hours, does not do so.

6. A concentration of 0·2 per cent. of the gas, acting for three hours, is sufficient to kill both fleas (adults and larvae), and rats.

7. Spraying with liquid Cyanide gives better results than does the dumping method, in that it tends to give a more uniform concentration throughout the area, although not ensuring this absolutely.

RECOMMENDATIONS

1. That a concentration of 0·3 per cent. of Hydrogen Cyanide, acting for a period of three hours, should be used.

2. That where match-boarding is present, one or two boards should, if possible, be removed, in order to allow the gas easy access into the cavity behind.

3. That where bunks with hollow metal frames are present, they should be taken to pieces, when this is practicable, and the tubular portions laid horizontally, so that the gas can penetrate easily into their interior. Or better, as a preventative, the ends of the tubing should be hermetically sealed, as illustrated on Plate II, fig. 1.

APPENDIX I

NOTE ON LETHAL CHAMBER AND CHEMICAL METHODS EMPLOYED

BY

W. H. ROBERTS, M.Sc., F.I.C.

CITY ANALYST

The material to be treated was placed inside a rectangular wooden chamber of internal dimensions 100 × 60 × 60 cms., closed with a lid, which, when clamped in position, rendered it air-tight. On the floor of the chamber and in one corner was placed a large porcelain dish containing dilute sulphuric acid. The lid was closed and the necessary quantity of potassium cyanide solution was run into the dish from a dropping funnel through a bent glass delivery tube passing through the wall of the chamber.

Two minutes later, sodium carbonate solution was run in from the same funnel, this being in order to expel all dissolved hydrogen cyanide gas from solution.

In the top of the opposite wall of the chamber was a glass delivery tube connected to a long length of india-rubber tubing. This and the inlet tube were now firmly clamped and the material left exposed for the time of the experiment.

In opening up the chamber the inlet tube was attached to a foot-bellows and slight pressure applied. Both clamps were now removed and air blown through for 15 minutes. At the end of this period the box could safely be opened.

To give a concentration of 0·3 per cent. HCN gas in the chamber, the following reagents were used :—

15 c.cs. H_2SO_4 (1 in 3 by volume).

3·2 grms. KCN (98 per cent.) dissolved in about 20 c.cs. of water.

followed by :—

20 c.c. of a 10 per cent. Na_2CO_3 solution.

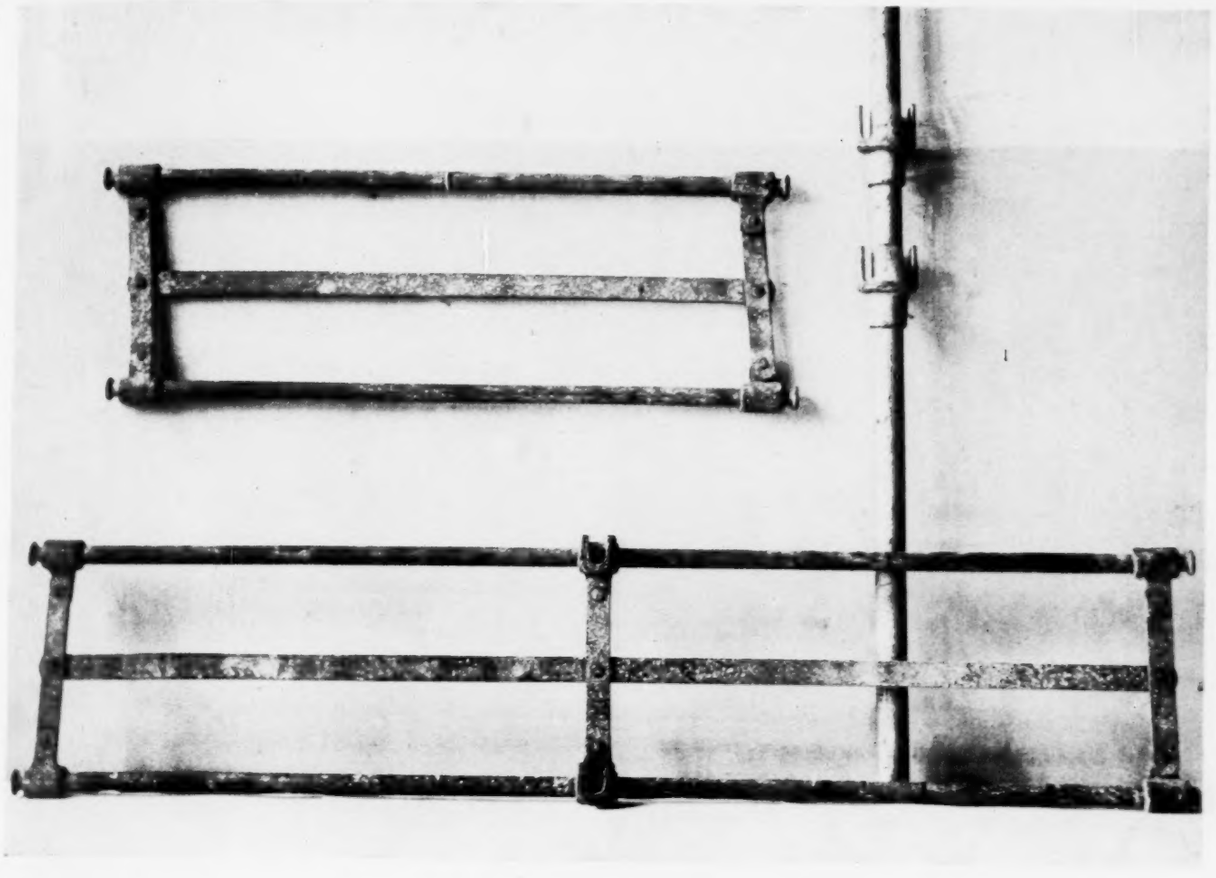

FIG. 1

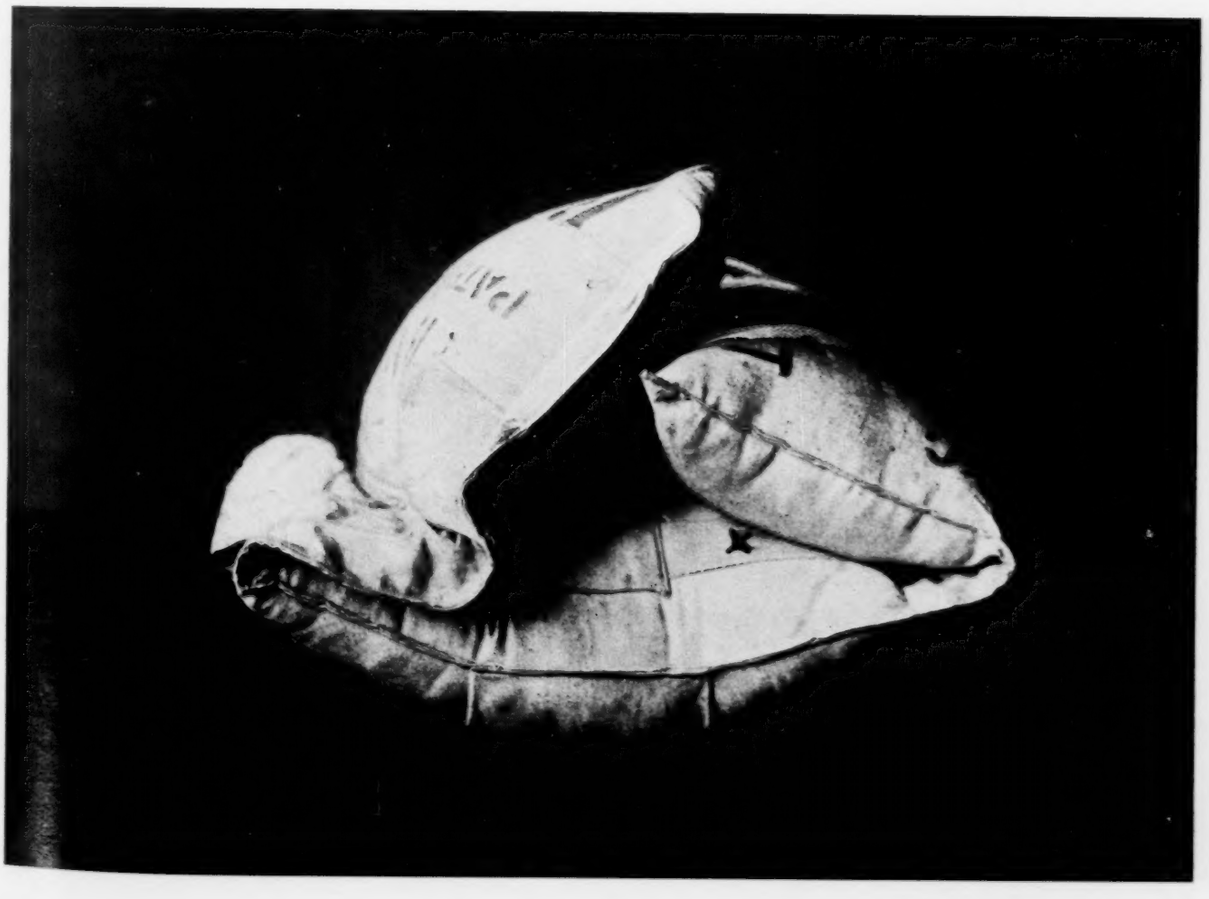

FIG. 2

C. Tinling & Co., Ltd., Imp.

NOTES ON CULICIDAE COLLECTED IN SIERRA LEONE, WITH DESCRIPTIONS OF A NEW SPECIES AND A NEW VARIETY

BY

A. M. EVANS

(*Received for publication* 12 *February*, 1925)

PLATE III

Professor Blacklock has recently made numerous collections of larvae of Culicidae from very varied situations at Daru, and on the Cape Lighthouse Peninsula, near Freetown. These situations included many ' small enclosed collections of water ' such as rot-holes in trees, and it may be interesting to compare these findings with those recorded by Macfie and Ingram (1923) from the Gold Coast.

The adults reared from these larvae were submitted to the writer for identification and have been found to comprise twenty-four species, seven of which do not appear to have been recorded from the Colony hitherto ; one species is an undescribed member of the *apicoargentea* series of ' *Stegomyia*,' and another a new variety of *Aedes* (*Aedimorphus*) *cumminsi*, Theo. The following is a list of the species contained in the collection ; the material from Daru was collected in the latter half of September, 1924, and that near Freetown on the 17th and 18th August in the same year.

The types and co-type specimens described in this paper are in the collections of the Liverpool School of Tropical Medicine.

Anopheles costalis, Loew.

Daru : Stream, 1 ♂ ; ' Swamp A,' 14 ♂♂, 12 ♀♀ ; Stream to ' Swamp B,' 1 ♀ ; Moa River, 6 ♂♂, 2 ♀♀ ; Hospital drain area, 1 ♂ ; Cape Lighthouse Peninsula, Freetown : Rock-pool, 1 ♀.

Anopheles nili, Theo.

Moa River, Daru, 14 ♂♂, 12 ♀♀.

Professor Blacklock observed that during the short period of investigation this species did not enter houses frequently. The

following observation tends to show a striking contrast in this respect between *A. nili* and *A. costalis*. He noted that in a native's house that was only fifty yards away from the edge of the Moa river, where *A. nili* was breeding in large numbers, only one adult specimen of this species was to be found. At the same time more than one hundred *A. costalis* were captured in this house, although its breeding-ground was ' in a marsh (Swamp A) further away.'

Anopheles mauritianus, Grandpré.

' Swamp B,' Daru, 1 ♀.

Anopheles umbrosus, Theo.

' Swamp A,' Daru, 1 ♀.

Anopheles rhodesiensis, Theo.

Rock-pool, Cape Lighthouse Peninsula, near Freetown: 4 ♂♂, 6 ♀♀.

Uranotaenia fusca, Theo.

Daru: stream to ' Swamp B,' 3 ♂♂, 3 ♀♀; tree-hole, 1 ♀.

Uranotaenia nigripes, Theo.

Daru: in a pineapple found in an empty bungalow, 1 ♂, 2 ♀♀; in a tree-root in ' native S.M.'s yard,' 1 ♀.

Mimomyia hispida, Theo.

' Hospital drain area,' Daru, 2 ♂♂, 1 ♀.

Aedes (*Stegomyia*) *argenteus*, Poiret.

Daru: latrine washing-bucket, 1 ♀; Cape Lighthouse Peninsula, Freetown; rock-pool, 1 ♀; tree-hole, 3 ♂♂, 1 ♀.

Aedes (*Stegomyia*) *africanus*, Theo.

Daru: Between forks of three-stemmed tree at ground level, 1 ♂, 4 ♀♀; tree-hole, 2 ♂♂; banana tree, near bungalow, 1 ♂, 3 ♀♀; stream, 1 ♂.

Aedes (*Stegomyia*) *simpsoni*, Theo.

Daru: hole in root of tree, in ' native S.M.'s yard,' 1 ♀.

Aedes (*Stegomyia*) *apicoargentea*, Theo.

Daru: stream near river, 1 ♂, 1 ♀; latrine washing-bucket, 3 ♀♀.

***AEDES (STEGOMYIA) BLACKLOCKI*, sp.n. (Pl. III, fig. 1).**

FEMALE.

Head and *palpi* with black and silvery-white scales arranged as shown in figure. *Thorax.* Mesonotum with silvery-white anterior patch formed of broad, flat scales in front and narrow curved scales behind. Middle line (largely denuded in the type) of narrow-curved, pale-yellow scales broadening posteriorly, the scales becoming silvery and forking to form two lines surrounding the ante-scutellar space; large paired silvery spots formed of broad curved scales; smaller silvery spots over wing roots of long curved scales; the scales forming the paired posterior lines very pale yellow, narrowly spindle-shaped. Scutellum with a few black scales behind the silvery ones on the median lobe, and one or two black scales internally on the right lateral lobe. Pleurae without lower mesepimeral bristles, a large patch of flat silvery scales on the upper part of the mesepimeron.

Abdomen. Dorsum of third segment with silvery-white scales forming an irregular and asymmetrical basal band. Fourth and fifth segments with well-developed silvery basal bands. Sixth and seventh segments with broad basal areas of silvery scales reaching to their distal borders in the middle. Third to seventh segments with large, basal, lateral, rectangular silvery spots, and ventrally with narrow basal silvery bands.

Legs. Front femora with a narrow line of white scales on the basal two-fifths anteriorly, and a line of silvery-white scales at the outer third, internally, not extending to the apex. Mid-femur with basal white spot; on the external face a median silvery spot, and a broad, apical, silvery patch continuous with a narrow, internal line of white scales extending backwards for nearly one-third the length of the segment. Hind femur creamy white at base beneath, with a conspicuous, silvery-white apical spot and a small, sub-median, external, silvery stripe. Front tibia with a narrow, basal, silvery-white ring, broadest beneath, mid tibia with a white basal patch beneath, hind tibia with a white spot near the base externally and a creamy-white stripe at the base beneath. Front tarsi with narrow, white bands on first two segments; mid tarsi with a basal, white band on the first segment, second segment creamy-white with a narrow, apical, black ring; hind tarsi with basal, white rings

about one-fourth to one-fifth the length of the segments, fourth segment white with a narrow, apical, black ring, fifth segment with a very small basal white band. Wing with dense black scales; length: 3·5 mm.

MALE.

Palpi with silvery-white scales forming a ring at about the middle of the long segment, a small, dorsal, sub-basal patch; small spots beneath the bases of the last two segments; occiput with several golden, upright forked scales behind; coloration of thorax and abdomen as in the female; but legs with mid tibia entirely dark, hind tibia with external white spot continuous with ventral stripe, last hind tarsal segment with basal half white. *Hypopygium* (Plate III. fig. 2) as in *C.* (*Stegomyia*) *apicoargentea*, but lobe of side-piece very narrow and furnished with three long bristles.

Co-type ♂♂ (2) and type ♀ bred from larvae taken from a tree-hole, Daru, Sierra Leone, 24.ix.1924, by Professor B. Blacklock and 1 ♂ and 1 ♀ taken from a tree-hole on the Cape Lighthouse Peninsula, near Freetown, 18.ix.1924.

In the specimens from the neighbourhood of Freetown, the third abdominal segment is without a white basal band, in the male this segment is completely dark-scaled, but in the female one or two whitish scales occur in the basal region.

This species is obviously one of the *apicoargentea* series of *Stegomyia* (Edwards, 1925) and appears to come nearest to *A.* (*S.*) *poweri*, Theo., from which it differs in having:—the silvery margin to the eyes not interrupted by dark spots; the large silvery areas on the mesonotum very broadly oval, not crescent-shaped; the basal abdominal bands not dull white, but markedly silvery; the fourth hind tarsal segment not all white and the fifth not all black.

Aedes (*Finlaya*) *longipalpis*, Grünb.

Daru: banana fibre, Mailemma, 1 ♂; tree-root, 3 ♂♂, 1 ♀; hole in tree-root, 5 ♂♂; tree-hole, 1 ♂, 1 ♀; stream, 1 ♀.

Aedes (*Aedimorphus*) *apicoannulatus*, Edw.

Tree-hole, Cape Lighthouse Peninsula, Freetown, 1 ♂, 1 ♀.

Aedes (*Aedimorphus*) *domesticus*, Theo.

Daru : ' Swamp A,' 1 ♀ ; ' Swamp B,' 1 ♂, 1 ♀ ; Moa River, 1 ♂.

Aedes (*Aedimorphus*) *tarsalis*, Newst.

Daru : tree, 1 ♂, 1 ♀ ; Moa River, 2 ♂♂ ; ' Swamp A,' 8 ♂♂, 18 ♀♀ ; ' Swamp B,' 3 ♂♂, 7 ♀♀ ; stream to ' Swamp B,' 1 ♂.

AEDES (*AEDIMORPHUS*) *CUMMINSI* var. *DARUENSIS*, n.var.

This variety differs from typical *A. cumminsi* as follows :— *Head* with the narrow curved scales creamy white. *Mesonotum* with very pale, brassy, rather long, narrow-curved scales and with short, dark-brown, almost hair-like scales concentrated in certain areas as follows :—a narrow, median stripe extending from the anterior border for about two-thirds the length of the mesonotum ; a pair of broadly ovate patches just in front of the sutures ; an inner and outer pair of stripes extending from the ante-scutellar region to beyond the posterior extremity of the middle stripe. *Abdomen* with small, but well-defined median, basal, pale spots on the third to seventh segments. Tibiae with well-marked white, apical spots, femora with apices narrowly pale. *Male hypopygium*—clasper as shown in Plate III, fig. 3.

Type ♂ and type ♀ reared from larvae, Moa River, Daru, Sierra Leone, 18.ix.1924, Professor Blacklock. One other female from the same locality.

The abdomen of the male is greatly contracted so that it is impossible to see whether median spots are present or not.

Mr. F. W. Edwards has kindly examined the type ♀ and tells me that though it seems near the var. *mediopunctata* of *cumminsi*, there are differences, and that in certain aspects it approaches *A.* (*Aedimorphus*) *caliginosus*.

Culex decens, var. *invidiosus*, Theo.

Daru : tree-hole, 1 ♂, 1 ♀ ; ' Swamp A,' 28 ♂♂, 42 ♀♀ ; ' Swamp B,' 1 ♀ ; ' hospital drain area,' 30 ♂♂, 49 ♀♀. Cape Lighthouse Peninsula, Freetown : rock-pool, 1 ♀.

Culex annulioris, Theo.

Daru : ' Swamp A,' 1 ♂ ; latrine washing-bucket, 1 ♂.

Culex thallassius, Theo.

Rock-pool, Cape Lighthouse Peninsula, Freetown, 16 ♂♂, 17 ♀♀.

Culex (*Culiciomyia*) *nebulosus*, Theo.

Daru: banana tree, Mailemma, 19 ♂♂, 14 ♀♀; old mortar, near river, 4 ♂♂; tree-holes, 8 ♂♂, 5 ♀♀; kerosene tin, 1 ♂; stream, 4 ♂♂, 1 ♀; stream near river, 1 ♂.

Lutzia tigripes, var. *fusca*, Theo.

Daru: swamps 'A' and 'B', 5 ♂♂, 5 ♀♀; 'hospital drain area,' 1 ♂, 8 ♀♀.

Toxorhynchites brevipalpis, Theo.

Daru: fork in orange tree, Mailemma, 1 ♀; 'hospital drain area,' 1 ♀.

Eretmapodites chrysogaster, Graham.

Daru: banana tree, Mailemma, 3 ♂♂, 6 ♀♀.

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PLATE III

EXPLANATION OF PLATE III.

Fig. 1. *Aedes* (*Stegomyia*) *blacklocki*, sp.n. ♀. × 50 about.

Fig. 2. *Aedes* (*Stegomyia*) *blacklocki*, sp.n. Male hypopygium. *l.*—lobe of side piece; *p.*—phallosome; *pl.*—lateral plate of anal lobe.

Fig. 3. *Aedes* (*Aedimorphus*) *cumminsi* var. *daruensis* var. n. Side-piece of male hypopygium.

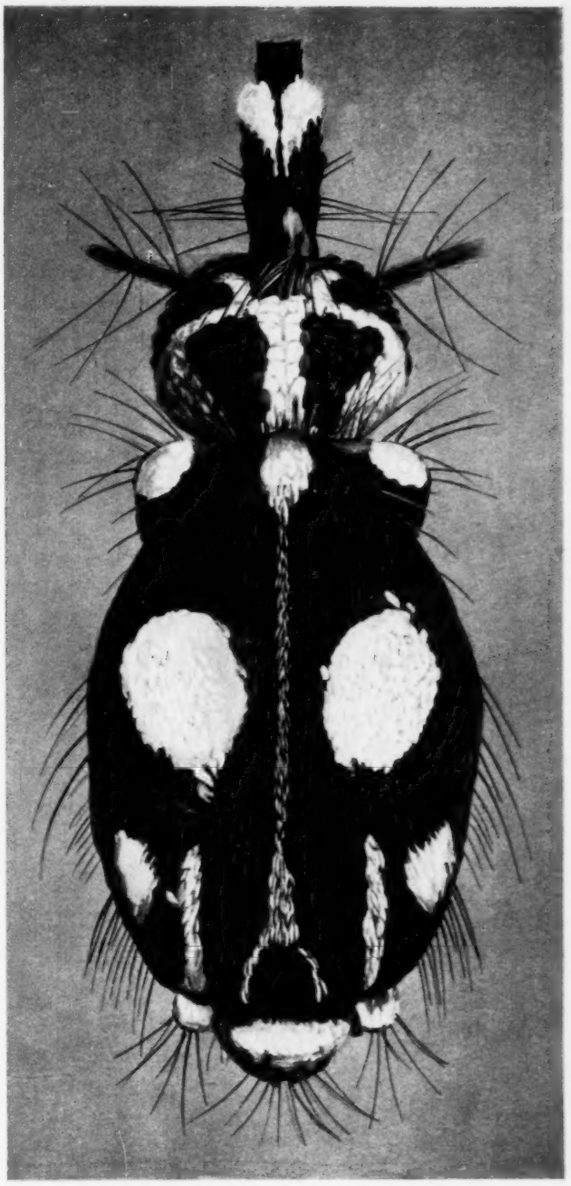


FIG. 1

FIG. 2

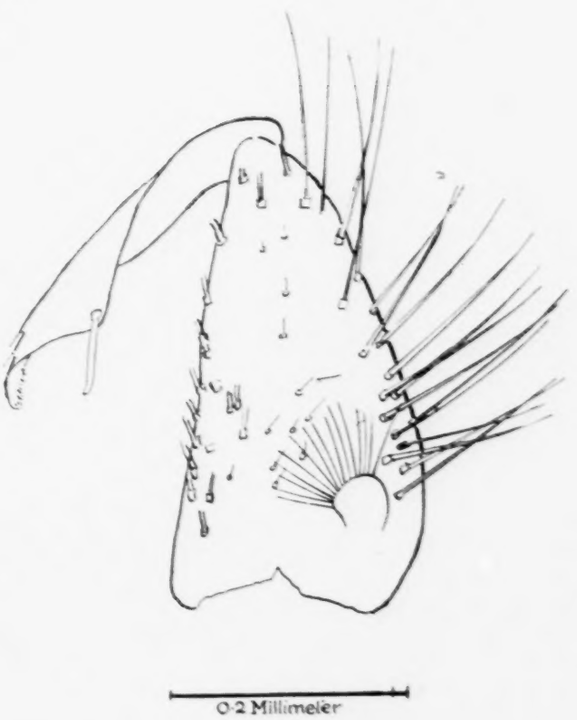


FIG. 3

C. Tinling & Co., Ltd., Imp.

A DISEASE OF FOWLS IN PALESTINE CHARACTERISED BY LEUCOCYTE INCLUSIONS

BY

S. ADLER

(*Microbiological Institute, Jerusalem*)

(*Received for publication* 13 *February*, 1925)

Mr. D. Ury, the director of an experimental poultry farm at Ben-Shemen, Palestine, called my attention to a disease of fowls on his farm which, although in its later stages it resembled spirochaetosis, was not amenable to treatment with atoxyl or neo-salvarsan. The disease is of considerable economic importance, as it attacks Rhode Island and Leghorns and hybrids of the above varieties with native fowls. Native fowls were not observed to be attacked. The first symptoms to be observed were depression and a refusal to take food ; later a tendency to stand still, and fever up to 110° F. ; finally the infected bird was unable to stand, diarrhoea with greenish stools developed and death took place from seven to fourteen days after the commencement of the first symptoms.

Examination of the blood revealed chromatic inclusions in the protoplasm of the leucocytes. The inclusions were of the following varieties :

(1) Minute granules of chromatin surrounded by a vacuole. The protoplasm of cells containing even a few of these forms was often markedly vacuolated.

(2) Small regular rings of chromatin.

(3) Spherical solid masses of chromatin.

(4) Irregular bacilliform masses of chromatin.

(5) Clusters of minute granules of chromatin not lying in vacuoles.

The above kinds of inclusions were also noted inside the nuclei of infected cells.

The normal polymorphs of fowls contain three types of granules.

(1) Spherical granules staining pale red with Romanowsky stains.

(2) Elongated fusiform granules usually staining like eosinophil granules with Romanowsky.

(3) Spherical granules staining deep blue with Romanowsky stains.

From the above types of granules the chromatic inclusions were readily distinguished, being stained with Giemsa like the nuclei of malaria parasites but more brilliantly.

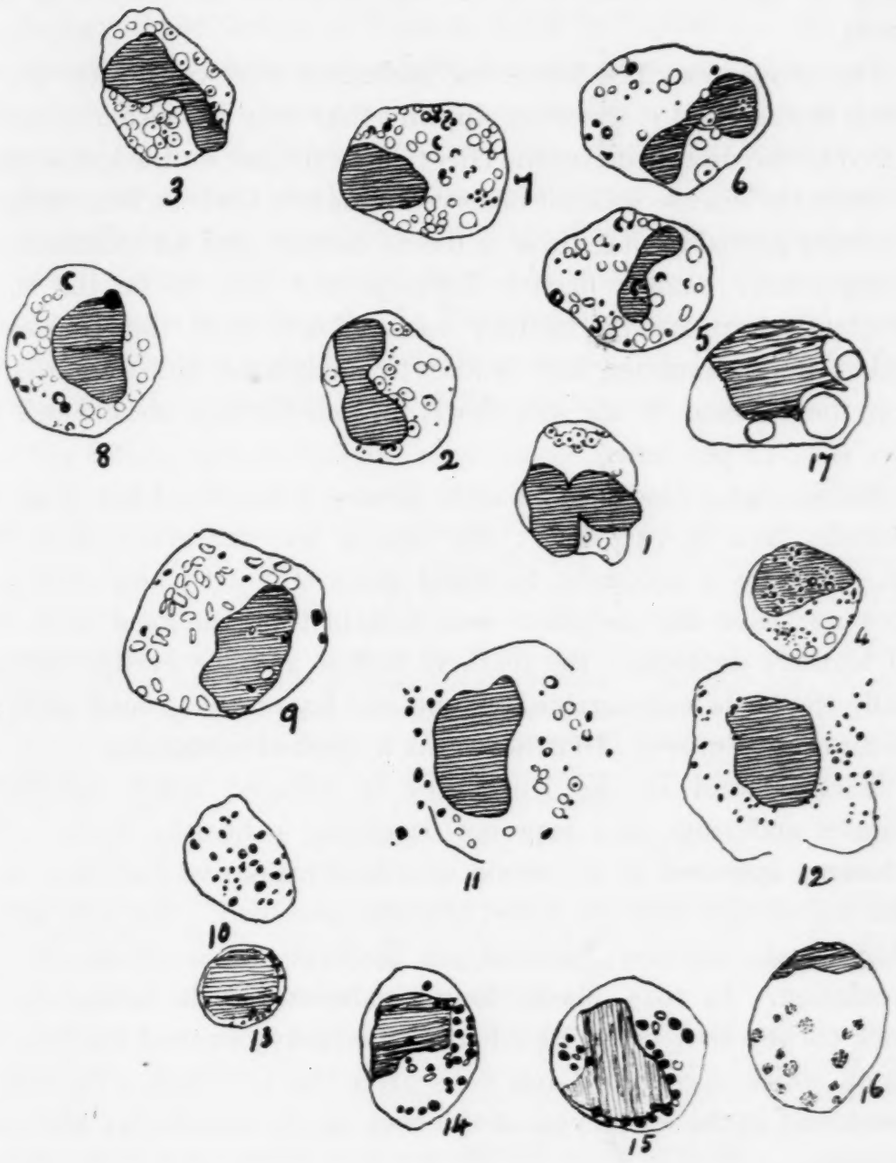
The number of inclusions in a leucocyte varied from one or two to very many ; in some instances the inclusions filled almost the whole cytoplasm of the leucocyte and appeared to escape into the general circulation by bursting the infected cell.

In addition to the inclusion in the leucocytes, small masses of protoplasm containing chromatic rings and solid spheres of chromatin were found in blood smears ; these masses are probably fragments of the cytoplasm of infected leucocytes.

All varieties of leucocytes except eosinophils and mast-cells contained the above-described inclusions, as many as 18 per cent. of the total leucocytes being infected. The nuclei of highly infected cells particularly of lymphocytes, tended to become degenerated and stained feebly with Giemsa in marked contrast to the brilliant staining of the inclusions. In highly infected lymphocytes the nucleus disappeared almost entirely and the cell stuffed with chromatic inclusions had a superficial resemblance to a Koch's blue body ; this form was found particularly in tissue smears. All stages between a slightly infected lymphocyte and the forms resembling Koch's blue bodies were found in tissue smears. A considerable number of the erythrocytes showed basophil staining of the protoplasm and rarely stippling resembling large Schüffner's dots.

Post-mortem the most striking changes were found in the liver and kidneys. The liver was enlarged and soft and studded with white patches ; on section, this organ showed fatty degeneration and infiltration ; the white patches were parts where fatty degeneration was most marked. In the kidneys, patches of necrosis were found.

Smears of the liver, lung, kidneys and spleen showed numerous leucocytes containing inclusions. Auto-erythro-phagocytosis, a phenomenon noted by Levaditi (1914) and by Macfie and Johnston



1 to 3.—Leucocyte with vacuoles some of which contain minute chromatic granules.

4 to 9.—Leucocytes with vacuoles and various forms of inclusions some of them apparently in the nucleus.

10.—Protoplasmic mass containing chromatic inclusions. From a lung smear.

11 to 12.—Leucocytes from which chromatic inclusions are escaping. Figure 11 from a case of *Leukaemia gallinarum*.

13.—An infected Lymphocyte.

14 to 15.—Leucocytes containing solid chromatic inclusions.

16.—A Leucocyte containing clumps of chromatic granules.

17.—An endothelial cell with three fragments of phagocyted erythrocytes.

× 1400.

(1914) in spirochaetosis of fowls was observed in smears of the organs.

The above described leucocytic inclusions were constantly found in this disease and it therefore appears that they are either casually related to the disease or are the effect of the disease on the leucocytes.

Since the disease was not observed in native fowls at Ben-Shemen it seemed probable that these acted as carriers and an examination of apparently healthy native fowls proved this to be the case. Twenty-five apparently healthy native fowls from the Jerusalem market were examined and leucocytic inclusions indistinguishable from those found in the sick fowls at Ben-Shemen were found in three (i.e., 12 per cent.).

Macfie (1914) described an acute disease of fowls in Eket, Nigeria, generally fatal in two days; the disease was characterised in the first stage by a tendency to stand stock-still with head and tail drooping, later the shoulders were hunched up, the head sunk, the tail feathers depressed, the feathers ruffled and the eyelids closed; finally the birds were unable to stand and lay on the ground without attempting to move. Diarrhoea was a marked symptom.

Macfie found in the leucocytes of infected fowls chromatic granules and rings of a type not occurring in healthy fowls. The inclusions appeared in the blood of a healthy native fowl five days after inoculation with the blood of a diseased fowl. The inoculated native fowl, however, showed no ill-effects as a result of the inoculation. In 1915, Macfie found inclusions in the leucocytes of a sick turkey in Accra. Blood from the turkey proved infective to a cock which succumbed ten days after the infection. Inclusions were found in the leucocytes of the cock on the fourth day after the infection.

The leucocytic inclusions described and figured by Macfie appeared to the writer identical with those found at Ben-Shemen. Blood smears from a healthy native fowl and from a sick fowl in Ben-Shemen were sent to Dr. W. Scott Macfie, of the Liverpool School of Tropical Medicine. Dr. Macfie kindly examined the slides and agreed that they contain inclusions indistinguishable from those he found in sick fowls in Eket. He further added that the disease in which he found the leucocytic inclusions was common in fowls in the Gold Coast and Nigeria.

In view of the similarity of the leucocytic inclusions and the pathology of the disease of fowls as found in Nigeria and the disease as found in Palestine, we consider the two diseases to be identical. The fact that the disease as it occurs in Ben-Shemen is of longer duration than the disease described by Macfie in Nigeria, is no evidence against the identity of the two diseases, for in spirochaetosis of fowls there is also an acute form of the disease lasting three to five days and a chronic form lasting about a fortnight after the appearance of spirochaetes in the blood. In Palestine both the acute and chronic form of spirochaetosis of fowls is present, but the chronic form lasting about a fortnight is much the commoner.

The following experiments were carried out :

(1) 4.11.24, blood (2 c.c.) from the wing vein of a healthy native fowl, No. 3, was injected intramuscularly into a healthy native fowl, No. 11. Leucocytic inclusions were found daily in No. 3, since 26.10.24. No. 11 had been examined daily, from 2.11.24 to 4.11.24, and no leucocytic inclusions were found. The total number of leucocytes in No. 11, at the time of the injection, was 8,500 per cmm. Leucocytic inclusions were found in the blood of No. 11, on 9.11.24. The first forms to appear were minute granules lying in vacuoles ; two days later chromatic rings and other forms appeared. On the day the inclusions appeared a leucocytosis of 20,000 per cmm. was observed ; the leucocytosis persisted for several days. Leucocytic inclusions persisted in the blood till 5.12.24. The fowl appeared healthy throughout an observation period of two months.

This experiment was repeated on healthy native fowls, No. 6, No. 7, No. 12, No. 13, with similar results. Chromatic inclusions in the leucocytes appeared five to six days after the injection, the first appearance of the inclusions being accompanied by a leucocytosis in one case, No. 12, up to 34,000 per cmm. Of the five healthy native fowls thus infected, one, No. 7, died ten days after the injection but the others appeared none the worse for the infection.

(2) Blood (2 c.c.) from the wing vein of No. 12 was injected into two healthy native fowls, No. 4 and No. 5, on 13.11.24. No. 4 and No. 5 had been under observation since 2.11.24 and no chromatic inclusions were found in their leucocytes. Chromatic inclusions were found in No. 4 on 19.11.24, and in No. 5 on 20.11.24. Neither of the two injected birds were affected by the injection.

(3) Blood (4 c.c.) from No. 3 was defibrinated and filtered through a Berkfeld filter. The filtrate was injected intramuscularly, on 13.11.24, into healthy native fowls, No. 8 and No. 9. These had been under observation since 2.11.24 and their leucocytes appeared free from the above described chromatic inclusions ; inclusions appeared in the leucocytes of No. 8 on 19.11.24. The bird died on 30.11.24 and smears of the blood and organs showed numerous leucocytic inclusions. Inclusions appeared in the leucocytes of No. 9 on 18.11.24, but no pathological results were noted as a result of the injection. It appears that the minute granules lying in vacuoles are infective, for the other forms of inclusions are too large to pass through a Berkfeld filter.

(4) Blood (2 c.c.) from No. 4 was injected into two healthy Leghorn cocks, No. 26 and No. 27, on 19.12.24. The leucocyte count of No. 26, at the time of the

experiment, was 6,400 per cmm. Chromatic inclusions were found in the leucocytes on 24.12.24. A leucocytosis of 18,000 per cmm. was noticed on the previous day, 23.12.24. Till 26.12.24 the only forms of inclusions noted in the leucocytes were minute granules lying in vacuoles; on 28.12.24 the other forms appeared. The bird was noticed to be ill on 24.12.24. It refused food and stood perfectly still; the temperature rose to 110° F. On 30.12.24 diarrhoea was noticed, the stools being greenish and on microscopical examination being found to contain numerous fat globules. Death took place on 31.12.24. Post mortem: the liver was found to be soft and fatty. Blood smears and organ smears showed numerous chromatic inclusions in the leucocytes.

In No. 27, leucocytic inclusions appeared in small numbers on 25.12.24. The leucocyte count on 19.12.24, the day of the injection, was 8,510 per cmm., and it rose to 14,500 per cm. on 25.12.24. The temperature rose to 110° F. on 24.12.24. The bird appeared ill from 24.12.24 till 30.12.24, and then recovered. Chromatic inclusions were present in small numbers in the leucocytes till 18.1.25.

(5) Highly infected blood (1 c.c.) from No. 12 was injected intramuscularly into three pigeons on 13.11.24. Chromatic inclusions such as described above were never found in the leucocytes of the pigeons during an observation period of six weeks.

Observations on healthy native fowls whose leucocytes contained chromatic inclusions showed that the inclusions persisted in the blood during a period varying from one to seven weeks. During this period crises of leucocytosis lasting one to two days were noted at irregular intervals. The leucocyte count rose to 34,000 per cmm. in one case. In this connection it is interesting to note that a blood smear from a sick fowl which died at Ben-Shemen was sent by Mr. D. Ury to the laboratory and a diagnosis of leukaemia was established; chromatic inclusions were found in the leucocytes, but whether the inclusions were aetiologically related to the leukaemia or whether, as is more probable, the case was a mixed infection of leukaemia and the disease described by Macfie, it is impossible to say, as the author could not find any other cases of *Leukaemia gallinarum* in Ben-Shemen or in Jerusalem. It seems unlikely that the leucocytic inclusions are related to *Leukaemia gallinarum* since, according to Ellerman and Bang (1908), the latter disease has an incubation period of one to two months.

THE RELATIONSHIP OF THE LEUCOCYTIC INCLUSIONS TO THE DISEASE

The constancy with which the leucocytic inclusions are found in the disease and the fact that the inclusions appear regularly in the blood of inoculated fowls, led Macfie to conclude that the inclusions are true parasites belonging probably to the Chlamydozoa and are

causally related to the disease. The above observations support Macfie's view.

That the leucocytic inclusions are not products of cell degeneration is proved by the fact that in highly-infected cells, particularly in lymphocytes, they may be so numerous as to exceed in volume the nucleus of the host cell. Moreover, with Giemsa they stain more brightly than the nucleus of the host cell.

DISTRIBUTION OF THE DISEASE

Since the disease is common in Nigeria and the Gold Coast and is present in Palestine, it seems probable that it is also present throughout the whole of North-west Africa and throughout the whole of North Africa and Egypt, but has hitherto escaped attention owing to its clinical resemblance to spirochaetosis.

TREATMENT

Atoxyl by mouth and neo-salvarsan intramuscularly, did not cause the disappearance of the leucocytic inclusions in healthy native fowls or in sick fowls from Ben-Shemen, and produced no effect on the course of the disease in the latter.

Injections of Bismuth Sodium tartrate (to which fowls are remarkably tolerant, 0·8 gms. per kilo-body-weight producing no ill-effects) also proved useless. The above therapeutic tests suffice to differentiate the disease from spirochaetosis, for spirochaetosis of fowls in Palestine as elsewhere yields readily to treatment with atoxyl or neo-salvarsan and 0·03 gms. per kilo-body-weight of bismuth sodium tartrate was found to be sufficient to cure fowls of spirochaetosis in Jerusalem.

TRANSMISSION

Native fowls from the Jerusalem market and diseased fowls from Ben-Shemen were examined for ectoparasites; *Mallophaga* sp. were observed and *Argas persicus* was found to be very common; the experimental farm at Ben-Shemen was found to be heavily infested with *Argas persicus*. It was expected that *Argas persicus* would

prove to be the carrier and the following experiments were carried out :—

(6) Fifty specimens of *Argas persicus*, taken from the farm at Ben-Shemen, were macerated in 10 c.c. saline. After maceration the resulting brown fluid was injected intramuscularly into four native fowls. The injected fluid acted as a strong local irritant and also produced general toxic results and a marked leucocytosis. The fowls appeared depressed for several days after the injection and one died three days later. The other three recovered and none showed the typical chromatic inclusions in their leucocytes during an observation period of two weeks and none developed spirochaetosis.

(7) Three batches, each of ten specimens of *Argas persicus*, which had been kept in the laboratory several months without a feed, were allowed to bite but not to complete a feed on an infected fowl, No. 4, whose blood contained all the above described varieties of leucocytic inclusions ; the ticks were then allowed to bite three native fowls, five, twelve, and twenty days later. In no case did the chromatic inclusions appear in the leucocytes during an observation period of three weeks.

(8) A native fowl whose blood showed a natural infection of the above-described leucocytic inclusion was placed in a cage with nine other native fowls whose blood, at the time (24.10.24), appeared free from the inclusions. The birds were not allowed out of the cage. A month later all were examined and the leucocytic inclusions were found in six.

The above experiments are, however, not sufficient to exclude the probability of *Argas persicus* being the carrier of the disease. Even Experiment No. 8 cannot be regarded as conclusive, for although care was taken to exclude *Argas persicus*, yet this parasite is so common in Palestine that its absence from the cage for a whole month in Experiment No. 8 cannot be guaranteed.

I have to thank Mr. D. Ury, of Ben-Shemen, for supplying me with material ; Dr. A. Felix, Pathologist to the Rothschild Hospital, Jerusalem, for kindly allowing me the use of his laboratory ; and Dr. W. Scott Macfie, for kindly examining blood smears.

SUMMARY AND CONCLUSIONS

A disease of fowls in Palestine characterised by various forms of chromatic inclusions in the leucocytes is described.

The inclusions appear to be identical with the leucocytic inclusions described and figured by Macfie from Eket, Nigeria.

The disease in Palestine is considered to be the chronic form of the disease described by Macfie.

The disease can be transferred to healthy fowls by blood inoculation.

The inclusions appear five to six days after the inoculation of infected blood.

The inclusions are considered to be true parasites belonging to the Chlamydozoa.

Transmission experiments with *Argas persicus* were unsuccessful, but the experiments are not conclusive.

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MISCELLANEA

CEYLON: PARASITE AND SPLEEN RATES. PARASITE RATIOS

The following data have been constructed from tables kindly sent to me by Mr. H. F. Carter, Malariologist, Ceylon :—

CEYLON (9 Provinces).

Spleen Rate

	Children (56372) %
Average	14
Maximum	56
Minimum	1

Parasite Rate

	Children (4647) %
Average	13
Maximum	29
Minimum	2

Parasite Ratios (1206)

	Average %	Maximum %	Minimum %
Malignant tertian	11	17	3
Simple tertian	61	85	57
Quartan	28	43	11
	100		

ANURADHAPURA LOCAL BOARD AREA (Ceylon).

Spleen Rate

	Children (661) %	Adults (1135) %
Average	50	30
Maximum	67	45
Minimum	31	21

Parasite Rate

	Children (300) %	Adults (410) %
Average	41	16
Maximum	85	47
Minimum	11	4

Parasite Ratios (209)

	%
Malignant tertian	10
Simple tertian	44
Quartan	46

J. W. W. STEPHENS

THE HOOKWORMS OF MAN IN SIERRA LEONE

4,305 hookworms were obtained from thirty-eight prisoners, treated by Dr. J. Wood, W.A.M.S., in the Freetown Jail, and from nine post-mortems. All the cases were natives of Sierra Leone.

Only two species, *Necator americanus* and *Ancylostomum duodenale* were found.

Of the total number of hookworms examined 3,929, i.e., 91·3 per cent., were *N. americanus* and 376, i.e., 8·7 per cent., were *A. duodenale*. The largest number of hookworms found in a single case was 483.

	N. Americanus.	*A. duodenale.*
Average number of hookworms per case.....	83·6	8
Highest number found in one case	483	196
Number of females in total number examined	2931	192
Number of males in total number examined	998	174

In *N. americanus* females were about three times as numerous as males, while in *A. duodenale* the sexes were about equal in number. Of the 376 specimens of *A. duodenale*, 302 were recovered from two post-mortems on natives from Rotifunk, in the interior. Excluding these two cases *A. duodenale* formed less than 2 per cent. of the total number of hookworms examined.

The presence of *A. duodenale* in Sierra Leone, both in man and in the civet cat, is of great interest, for according to Darling (1920) this parasite has not been recorded from man in Equatorial Africa. Darling states that *A. duodenale* is the only hookworm recorded from man in North Africa and *N. americanus* the only one recorded from man in Equatorial and South Africa. Sierra Leone is, evidently, intermediate between the zones of distribution of *A. duodenale* and *N. Americanus*, the latter hookworm largely predominating.

S. ADLER

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CESTODES FROM EAST AFRICA

The following from a guinea fowl in Kenya Colony, East Africa, and sent by Mr. Brassey Edwards, M.R.C.V.S., were identified :—

Cotugnia digonophora (Pasq., 1890).
Metroliasthes lucida (Ransom, 1900).

M. J. W. WALKER

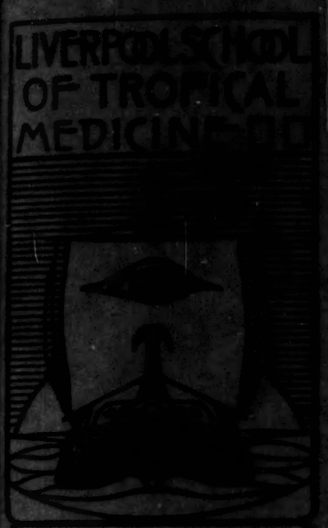
FASCIOLA HEPATICA

'7. *What causes* flounders, real little *flat fish*, brown on one side, white on the other, mouth side-ways, with tail, fins, and all, *leaping alive*, in the *inside* of a rotten sheep's, and every rotten sheep's *liver*?' ('Rural Rides,' William Cobbett, 1853, p. 281.)

J. W. W. STEPHENS

UNIVERSITY OF LIVERPOOL

y 16, 1925 XIX, No. 2



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CORRIGENDA

Vol. XVIII, No. 4

Page 626. W. Rees Wright. 'On the Hibernation of Adult Mosquitos.' Paragraph 2, lines 3, 4, 5, read :—

'. . . lofts or barns, when these communicate with a building containing animals. The insect flies spontaneously . . . '

ADDENDA. It was found, in the examination of some farms in the Wirral, during the winter of 1924-25, that mosquitos were most numerous in stables, and in lofts over shippons. *Anopheles maculipennis* ♀♀ were the predominant insects, though in the lofts *Culex pipiens* ♀♀ were nearly as abundant. A re-examination of the buildings late in April indicated a great reduction in the numbers present ; it is possible that they had by then left shelter to oviposit.

In these farms the lofts and stables were, as far as could be determined without a thermometer, decidedly warmer than the other buildings.

In North Carnarvonshire, *Anopheles* ♀♀ were very numerous in all buildings examined at the end of April, while by the end of May they had become very scanty ; for example, on a farm near Carnarvon, several hundred insects were easily collected on April 17th and the succeeding three days, while only four could be obtained, after a careful search, on 27th May.

Vol. XIX, No. 1

Page 69. Under *Proleptus obtusus* Duj. 1845 read :—

Material :—Numerous specimens collected from dog fishes in Ceylon and South Africa.

Page 120. For *Uranotaenia nigripes* Theo.
Read *Uranotaenia ornata* Theo.

ON A COLLECTION OF ACANTHOCEPHALA IN THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

BY

T. SOUTHWELL

AND

J. W. S. MACFIE

(*Received for publication* 11 *March*, 1925)

TABLE OF CONTENTS

The examination of this small collection of Acanthocephala has led us to attempt a tentative classification of the numerous genera hitherto described. The classification is based largely on the published descriptions of various authors which, unfortunately, are sometimes incomplete in details, a knowledge of which would

have been of great assistance, since the simplified morphology of the Acanthocephala offers at best but few characters on which to base a classification, and even these are liable to variation. Our work has therefore been one of great difficulty, and the result leaves much to be desired. To classify the group satisfactorily it will be necessary to obtain a much larger collection of species than we have had at our disposal, and a more extensive knowledge of the life history of the various worms.

Amongst the somewhat unsatisfactory characters upon which it has been necessary to base classification, mention should be made of the following :—

(1) *Lemnisci.* Even in mature worms, the length of the lemnisci appears (at least in certain genera) to vary within rather wide limits ; the length also varies, of course, with age ; and, moreover, the length relative to the total length of the body varies somewhat with the state of contraction or relaxation of the worm. From a systematic point of view, therefore, account must be taken of the age of the specimen and of the degree to which it is contracted.

(2) *Testes.* In young worms the shape, size, and relative position of the testes may be quite different from what they are in the adult. Reference has been made to this fact in the description of *M. moniliformis.* The degree of contraction of the body may also alter to some extent the position of the testes in the body and their relationship to each other, and this should be taken into account in those cases in which the position of the testes is of systematic importance.

(3) *Prostatic glands.* No reliance can be placed on the appearance of the prostatic glands of young worms. In mature worms it is frequently extremely difficult to determine the number of prostatic glands, but as some authors attach great importance to it, we have been unable to avoid employing it as a diagnostic character (see ECHINORHYNCHIDAE). Moreover, our experience has convinced us that the shape and arrangement of the prostatic glands are by no means constant, and as diagnostic characters must not be pressed too far, only differences of considerable degree being significant.

(4) *Eggs.* Eggs taken from the body cavity may or may not be fully developed and therefore it is clearly unwise to describe the eggs from specimens obtained in this manner. We have frequently

observed notable differences to exist between the more and the less mature eggs in a single worm. As the characters of the eggs are occasionally of importance, however, and as usually the only eggs available for examination are those taken from the body of the worm, it is important to select for description none excepting those which appear to be mature, namely, those in which the three concentric membranes are clearly defined, and the ring of hooks on the embryo developed. As an aid to the recognition of mature eggs we may say that, so far as our experience goes, the embryos in them are of a brownish colour.

With reference to the retractibility of the proboscis, a distinction must be drawn between a retraction of the entire proboscis or ' proboscis-like structure ' within the anterior part of the body, and a retraction (invagination) of the proboscis within its sheath. In this paper a reference to the proboscis as being retractile means that it is capable of being invaginated into its sheath.

As we employ certain terms in a sense in which they are not used uniformly by other authors the following definitions must be given :—

(1) *Proboscis*. The proboscis, as usually understood, signifies the process at the anterior extremity of the body which is used as an organ of fixation, and which (excepting in *Apororhynchus hemignathi*) is armed with hooks. We consider that this structure is not always morphologically identical, and therefore we propose to limit the term ' proboscis ' to that part of the process at the anterior extremity of the body which lies anterior to the insertion of the proboscis-sheath, and to use the term ' proboscis-like structure ' when referring to the proboscis as understood colloquially. We cannot agree with Lühe and Van Cleave (1916) in considering this unreasonable because it involves the admission that in the genus *Gigantorhynchus* there is little or no true proboscis. On the contrary, we regard it as characteristic of the genus *Gigantorhynchus* that the proboscis is reduced, and maintain that the morphology of the ' proboscis-like structure ' of *G. echinodiscus*, and the forms of the hooks with which that structure is armed, afford strong support to the view that in this species the true proboscis is represented by only the one or two circles of large hooks at the anterior extremity.

(2) *Body*. We define the anterior limit of the body as being situated at the level of the insertion of the lemnisci. This is, of course,

a purely arbitrary definition which, however, we consider necessary for systematic purposes.

(3) *Neck.* Considerable importance is attached to the presence or absence of a neck in the Acanthocephala, and to the presence or absence of hooks on this neck, but there does not appear to us to be any general agreement as to what constitutes a neck, some authors using the term to indicate a zone, often devoid of hooks, at the base of the 'proboscis-like structure,' and others using it in a more restricted sense. We therefore propose to define the neck as being that part of the worm which lies between the base of the proboscis and the anterior extremity of the body, that is, between the level of the insertion of the proboscis-sheath and the level of the insertion of the lemnisci. Thus in the genus *Echinorhynchus* the proboscis, using the term in its colloquial sense, is entirely or almost entirely the true proboscis, in the genus *Gigantorhynchus* it is largely neck, whilst in the genus *Centrorhynchus* it is approximately half true proboscis and half neck.

Classification.—Westrumb, in 1821, briefly reviewed the earliest observations made on the Acanthocephala. The order **Acanthocephala** was established by Rudolphi, in 1809, the following being the characteristics assigned to it by him in 1819:—'Corpus teretiusculum, utriculare, elasticum. Proboscis seriatum uncinata retractilis. Individua alia mascula, alia feminea.' Rudolphi recognised one genus only, namely, *Echinorhynchus*, with the characters of the order. Diesing, in 1851, accepted Rudolphi's classification, recognising only the single genus *Echinorhynchus*, but considered the order **Acanthocephala** to be a tribe which he included in the sub-order **Aprocta.**

Cobbold, in 1879, erected the family ECHINORHYNCHIDAE to accommodate the single genus *Echinorhynchus*, but did not define its characters; and Leuckart, in 1886, used the family name ACANTHOCEPHALIDAE without stating either the characters of the family or the genera he proposed should be included in it, but apparently for the reception of the single genus *Echinorhynchus*.

The first important attempt to split up the Acanthocephala was made by Hamann, who, in 1892 and 1895, divided them into three families as follows:—

(1) ECHINORHYNCHIDAE. Body elongated, smooth. Proboscis-

sheath with double walls ; the proboscis-sheath receives the proboscis. Nerve ganglion in the proboscis-sheath, generally in its depth, centrally placed. Hooks chitinised only at their tips, and with a root-like process below.

Genus *Echinorhynchus ;* with the characters of the family.

(2) GIGANTORHYNCHIDAE. Large species with a segmented, flat, taenia-like body when alive. Hooks like those of *Taenia*, being entirely covered with chitin, and with two root-like processes. Proboscis-sheath muscular, inserted into the proboscis, and into which the proboscis cannot be retracted. Nerve ganglion situated behind the middle of the proboscis-sheath, lying laterally and eccentrically. The body cavity lined by a structureless membrane and traversed by oblique membranes. Lemnisci long coiled tubes with a central canal.

Genus *Gigantorhynchus ;* with the characters of the family.

(3) NEORHYNCHIDAE. Species which become sexually mature in the larval state. Proboscis-sheath a tube with a simple wall. In the skin, and in the lemnisci, are a few giant nuclei. Circular muscles very simply developed ; and the longitudinal muscles only present here and there.

Genus *Neorhynchus ;* with the characters of the family.

Since the publication of Hamann's classical work numerous authors have contributed to our knowledge of this interesting group of parasitic worms, amongst whom especial mention should be made of Lühe, Porta, Van Cleave, and Travassos.

The tentative classification which we propose is as follows. The species which we have had at our disposal are indicated in the body of the paper.

Phylum	NEMATHELMINTHES.
Order	*ACANTHOCEPHALA.*
Sub-order (1)	**Neoechinorhynchidea,** nom. nov.
Family (1)	NEOECHINORHYNCHIDAE Van Cleave, 1919.
Genera	*Neoechinorhynchus* Stiles and Hassall, 1905.
	Tanaorhamphus Ward, 1918.
	Octospinifer Van Cleave, 1919.
	Gracilisentis Van Cleave, 1919.
	Pandosentis Van Cleave, 1920.

Family (2)	QUADRIGYRIDAE Van Cleave, 1920.
Genus	*Quadrigyrus* Van Cleave, 1920.
Family (3)	APORORHYNCHIDAE Shipley, 1900.
Genus	*Apororhynchus* Shipley, 1900.
Sub-order (2)	**Gigantorhynchidea,** nom. nov.
Family (1)	GIGANTORHYNCHIDAE Hamann, 1892.
Genus	*Gigantorhynchus* Hamann, 1892.
Family (2)	OLIGACANTHORHYNCHIDAE, nom. nov.
Genera	*Macracanthorhynchus* Travassos, 1917.
	Oligacanthorhynchus Travassos, 1915.
	Prosthenorchis Travassos, 1915.
Sub-order (3)	**Echinorhynchidea,** nom. nov.
Family (1)	RHADINORHYNCHIDAE Travassos, 1923.
Genera	*Rhadinorhynchus* Lühe, 1911.
	Leptorhynchoides Kostylev, 1924.
	Arhythmorhynchus Lühe, 1911.
	Serrasentis Van Cleave, 1923.
	Telosentis Van Cleave, 1923.
Family (2)	CENTRORHYNCHIDAE Van Cleave, 1916.
Genera	*Centrorhynchus* Lühe, 1911.
	Mediorhynchus Van Cleave, 1916.
	Empodius Travassos, 1916.
Family (3)	CORYNOSOMIDAE, nom. nov.
Genera	*Corynosoma* Lühe, 1904.
	Bolbosoma Porta, 1908.
	Polymorphus Lühe, 1911.
	Filicollis Lühe, 1911.
	Tegorhynchus Van Cleave, 1920.
Family (4)	MONILIFORMIDAE Van Cleave, 1924.
Genus	*Moniliformis* Travassos, 1915.
Family (5)	ECHINORHYNCHIDAE Cobbold, 1879.
Genera	*Prosthorhynchus* Kostylev, 1916.
	Oligoterorhynchus Monticelli, 1914.
	Pomphorhynchus Monticelli, 1905.
	Acanthocephalus Koelreuter, 1771.
	Echinorhynchus Zoega, 1776.

PHYLUM NEMATHELMINTHES.

Order *ACANTHOCEPHALA*.

Nemathelminthes without a gut, and with a proboscis-like structure which is usually armed with hooks.

With three sub-orders.

KEY TO THE SUB-ORDERS OF THE ORDER ACANTHOCEPHALA.

1. Prostatic glands a single syncytial mass..... *Neoechinorhynchidea* (1)
   Prostatic glands not a single syncytial mass.....2
2. Proboscis reduced, not capable of being withdrawn into the proboscis-sheath..... *Gigantorhynchidea* (2)
   Proboscis well developed and capable of being withdrawn into the proboscis-sheath..... *Echinorhynchidea* (3)

Sub-order I. **NEOECHINORHYNCHIDEA,** nom. nov.

Proboscis usually short and sub-spherical. Proboscis-sheath (when present) a tube with a simple wall. Prostatic gland a single syncytial mass. Nuclei of sub-cuticle and lemnisci few and very large.

The order is divided into three families.

KEY TO THE FAMILIES OF THE ORDER NEOECHINORHYNCHIDEA.

1. With a proboscis armed with hooks.....2
   Without such a proboscis..... *Apororhynchidae* (3)
2. Body bearing spines on the anterior region..... *Quadrigyridae* (2)
   Body devoid of spines..... *Neoechinorhynchidae* (1)

Family (1) NEOECHINORHYNCHIDAE Van Cleave, 1919

Neoechinorhynchidea of small to medium size. Wall of proboscis-sheath a single layer of muscle. Central nervous system near base of proboscis-sheath. Body devoid of spines; spines or hooks on proboscis only. Nuclei of sub-cuticle and lemnisci extremely large, normally of fixed number and definite arrangement, the sub-cuticle with five in the mid-dorsal line of the body and one in the mid-ventral line near the anterior end, and the lemnisci with two in one lemniscus and a single one in the other. Testes elliptical, usually contiguous. Prostatic gland a single syncytial mass containing relatively few

giant nuclei. Eggs where known with three membranes, and without polar capsules. Parasitic* in fish and reptiles (turtles).

The family contains five genera.

KEY TO THE GENERA OF THE FAMILY NEOECHINORHYNCHIDAE.

1. Proboscis armed with 3 circles of hooks.....2
   Proboscis armed with more than 3 circles of hooks.....3

2. Proboscis armed with 3 circles of 6 hooks each..... *Neoechinorhynchus* (1)
   Proboscis armed with 3 circles of 8 hooks each..... *Octospinifer* (3)
   Proboscis armed with 3 circles of 12 hooks each..... *Gracilisentis* (4)

3. Proboscis several times longer than wide, armed with about 16 to 20 longitudinal rows each composed of about 10 hooks..... *Tanaorhamphus* (2)

   Proboscis short, cylindrical, armed with about 22 longitudinal rows each composed of about 4 hooks... *Pandosentis* (5)

With regard to the last two genera, Van Cleave (1923) states in his key to the genera of Acanthocephala that in the genus *Tanaorhamphus* the proboscis bears 'twenty or more circles of hooks,' and in *Pandosentis* 'eight circles of hooks.' We are unable to harmonise these statements with his earlier generic definitions which we give below.

Genus (1) *Neoechinorhynchus* Stiles and Hassall, 1905.

SYNONYMS :—*Echinorhynchus* Zoega, in Müller, 1776, in part.
Neorhynchus Hamann, 1892, preoccupied.
Eorhynchus Van Cleave, 1914.

Diagnosis.—Neoechinorhynchidae with short, globose proboscis armed with three circles of six hooks each. Terminal hooks conspicuously larger and heavier than those of remaining rows, and the only ones which bear conspicuous reflexed root-like processes. Each root a broad, flattened disc pyriform in surface view, usually approximately parallel to surface of proboscis wall. The thorn or hook proper attached at the apical or anterior end of the root, and appreciably longer than the root. Parasitic in fish and turtles.

Type species : *N. rutili* (Müller, 1780).

A single species belonging to this genus was found in the collection. This appeared to be a new species and is briefly described below.

* Unless otherwise stated the hosts given in this paper are those in whose alimentary canal the adult worms are found.

Neoechinorhynchus magnus, sp.n.

One immature female specimen only ; host unknown. Townsville, Queensland, Northern Australia. (Dr. P. A. Maplestone).

The specimen measured 90 mm. in length, and the maximum breadth was about 1·5 mm. The body is flattened and tape-like, the anterior extremity being much narrower than the posterior extremity ; the skin is slightly corrugated.

Proboscis. The proboscis is small, sub-globular, and armed, as is usual in the genus, with eighteen hooks in three rows, the anterior six being larger than the rest. The hooks of the terminal circle measure in length from 60μ to 71μ, those of the middle circle 30μ to 37μ, and those of the basal circle about 18μ.

Proboscis-sheath. This measures 0·5 mm. in length and the greatest breadth is 0·2 mm.

Lemnisci. These are slightly unequal in length and measure from five to six times the length of the proboscis-sheath.

The species differs from all other species of the genus in being very much longer.

Genus (2) *Tanaorhamphus* Ward, 1918.

Synonym :—*Neoechinorhynchus* Stiles and Hassall, 1905, in part.

Diagnosis.—Neoechinorhynchidae of small to medium size, with cylindrical proboscis several times longer than wide. Proboscis armed with about sixteen longitudinal rows of hooks. Rows frequently incomplete and imperfect. Prostatic gland of the type characteristic of the family. Parasitic in fish.

Type species : *T. longirostris* (Van Cleave, 1913).

Genus (3) *Octospinifer* Van Cleave, 1919.

Diagnosis.—Proboscis short, globose, usually slightly broader than long ; provided with three circles of eight hooks each. Hooks of terminal circle not much larger or stronger than hooks of middle circle and but little longer than the root-process. Testes elliptical, in contact with each other but not joined by a broad contact-surface. Prostatic gland not in direct contact with posterior testis. The two lemnisci dissimilar in nuclear content, one possessing two giant nuclei and the other a single one. Central nervous-system located at one side of the proboscis-sheath, near its base. Parasitic in fish.

Type species : *O. macilentus* Van Cleave, 1919.

Genus (4) *Gracilisentis* Van Cleave, 1919.

SYNONYM :—*Neoechinorhynchus* Stiles and Hassall, 1905, in part.

Diagnosis.—Neoechinorhynchidae of small size. Body proper unarmed. Proboscis provided with three circles of twelve hooks each. Each hook ensheathed in a prominent cuticular collar which permits only a small portion of it to protrude from the surface of the proboscis. Each hook of the terminal circle provided with a conspicuous root-process several times longer than the exposed portion of the spine. Root composed of a broad flat basal area which, by gradual diminution in size anteriorly, makes an ill-defined transition from thorn to root. Basal region of terminal roots frequently slightly indented. Hooks of middle circle similar in general form to those of terminal circle, except that root-processes are shorter and less easily observed. Basal hooks without recurved roots. Parasitic in fish.

Type species : *G. gracilisentis* (Van Cleave, 1913).

Genus (5) *Pandosentis* Van Cleave, 1920.

Diagnosis.—Neoechinorhynchidae, with the characters of the family, except for the variation in arrangement of giant nuclei within the sub-cuticle. These do not always lie in the sagittal plane, as in representatives of all the other genera previously included in this family, but are frequently lateral in distribution. Body proper small, devoid of spines. Proboscis short, cylindrical, provided with more than three circles of hooks. Boundary between root and thorn usually not sharply marked. Arrangement of male genital organs as in members of the genus *Gracilisentis*. Testes elliptical, contiguous. Prostatic gland a rounded syncytial mass immediately following the posterior testis, with its posterior boundary indented for the reception of the reservoir of the prostatic gland. Prostatic gland in the only known species contains sixteen giant nuclei. Central nervous system at base of proboscis-sheath. Retractors of sheath emerge from the sheath at its posterior extremity on dorsal and ventral surfaces. Lemnisci not as long as the proboscis-sheath. Parasitic in fish.

Type species : *P. iracundus* Van Cleave, 1920.

Family (2). QUADRIGYRIDAE Van Cleave, 1920.

Neoechinorhynchidea of medium size. Anterior body region provided with cuticular spines. Proboscis-sheath enclosed by a single muscular wall. Central nervous system located near the base of the proboscis-sheath. Subcuticular nuclei in anterior region elliptical, in sagittal plane ; in remainder of body a few large, branched nuclei laterally arranged. Parasitic in fish.

The family contains only a single genus.

Genus *Quadrigyrus* Van Cleave, 1920.

Diagnosis.—Quadrigyridae of medium size. Proboscis armed with four circles of hooks. Anterior surface of body usually provided with four circles of cuticular spines. Subcuticular nuclei of two types ; those of anterior part of body ovoid giant nuclei, dorsal and ventral in location ; those in remainder of body a large, central elongated mass, from which heavy lateral projections are given off, usually lateral in distribution. Proboscis-sheath provided with a single, heavy muscular wall. Central nervous system located near posterior extremity of proboscis-sheath. Parasitic in fish.

Type species : *Q. torquatus* Van Cleave, 1920.

Family (3) APORORHYNCHIDAE Shipley, 1900.

Neoechinorhynchidea of short form with the body divided into three well-marked regions. The head (proboscis) is pitted but not armed with hooks. There is no eversible introvert, no proboscis-sheath and no armature of hooks. The sub-cuticle and lemnisci have a few giant nuclei, and the lemnisci are long and coiled. Parasitic in birds.

The family contains only a single genus.

Genus *Apororhynchus* Shipley, 1900.

SYNONYM :—*Arhynchus* Shipley, 1896.

With the characters of the family.

Type species : *A. hemignathi* Shipley, 1896.

With regard to this species, Marval (1905) writes : ' Nous nous permettrons donc, maintenant, de considérer *Arhynchus hemignathi*

comme un *Neorhynchus*, endoparasite comme tous les Acanthocéphales, sans exception, et privé de rostre soit accidentellement ce qui est probable, soit à la suite de longues modifications telles que celles qui se produisent chez l'*Echinorhynchus filicollis* et *sphaerocephalus*, lors de la transformation du rostre en bulle.'

Sub-order II. GIGANTORHYNCHIDEA

Proboscis reduced, often composing only a small part of the proboscis-like structure; proboscis-sheath with a thick muscular wall into which the proboscis (when present) cannot be retracted, the proboscis-sheath being inserted near the anterior extremity. Neck present. Nuclei of the sub-cuticle and lemnisci relatively small and numerous. Prostatic glands not a single syncytial mass. Parasitic in mammals and birds.

The order is divided into two families.

KEY TO THE FAMILIES OF THE SUB-ORDER GIGANTORHYNCHIDEA.

Proboscis greatly reduced, represented by only one or two transverse rows of large hooks at the anterior extremity of the proboscis-like structure. Neck armed with numerous small hooks..... *Gigantorhynchidae* (1)

Proboscis sub-spherical, armed with 5 or 6 transverse rows of hooks. Neck unarmed..... *Oligacanthorhynchidae* (2)

Family (1) GIGANTORHYNCHIDAE Hamann, 1892.

Gigantorhynchidea of large size. Body apparently segmented. Proboscis rudimentary, represented by one or two transverse rows of hooks. Hooks with double roots. Neck armed with numerous small hooks. Lemnisci filiform, with numerous nuclei. Testes ellipsoidal, elongated, situated posteriorly. Prostatic glands subspherical. Parasitic in mammals.

The family contains only a single genus.

Genus *Gigantorhynchus* Hamann, 1892.

SYNONYM :—*Echinorhynchus* Zoega, 1776, in part.

With the characters of the family.

Type species : *G. echinodiscus* (Diesing, 1851).

Family (2) OLIGACANTHORHYNCHIDAE, nom. nov.

Gigantorhynchidea of small to large size. Body more or less rugose. Proboscis sub-spherical or nail-like, armed with five or six transverse rows of hooks. Hooks (excepting those at the base) with double roots. Neck short, unarmed. Testes ellipsoidal or cylindrical. Prostatic glands eight, ellipsoidal or nail-like. Parasitic in mammals and birds.

The family contains three genera.

KEY TO THE GENERA OF THE FAMILY OLIGACANTHORHYNCHIDAE.

1. Sexual dimorphism well marked; females very large and spirally coiled, males small, comma-shaped. Lemnisci relatively short and flat. Testes situated some distance anterior to the prostatic glands. Genital organs of the male occupying two-thirds of the body cavity..... *Macracanthorhynchus* (1)

   Sexual dimorphism not well-marked. Lemnisci narrow and cylindrical.....2

2. Genital organs of the male situated posteriorly and occupying about a quarter of the body cavity..... *Oligacanthorhynchus* (2)

   Genital organs of the male occupying two-thirds or more of the body cavity..... *Prosthenorchis* (3)

Genus (1) *Macracanthorhynchus* Travassos, 1917.

SYNONYMS :—*Echinorhynchus* Zoega, 1776, in part.
Gigantorhynchus Hamann, 1892, in part.

Diagnosis.—Sexual dimorphism well-marked; females very large and spirally coiled, males small, comma-shaped. Proboscis very large. Lemnisci rather short and flat, extending backwards to the anterior testis. Genital organs of the male occupying two-thirds of the body cavity. Testes long, cylindrical. Parasitic in mammals.

Type species: *M. hirudinaceus* (Pallas, 1781).

A single species belonging to this genus was found in the collection, namely :—

Macracanthorhynchus hirudinaceus (Pallas, 1781).

SYNONYMS :—*Taenia haeruca* Pallas, 1766, preoccupied, in part.
Taenia hirudinacea Pallas, 1781.
Echinorhynchus gigas Bloch, 1782.
Gigantorhynchus gigas of Hamann, 1892.
Gigantorhynchus hirudinaceus of Porta, 1908.

Six females and five males; host unknown. Hong Kong, January, 1914 (Dr. Bell). Also one male and one female; host unknown. Kindly lent by A. W. Noel Pillers, F.R.C.V.S., D.V.S.M.

The largest female measured 532 mm. in length, and 9 mm. in greatest breadth. The largest male measured 80 mm. in length, and 6 mm. in greatest breadth.

Genus (2) *Oligacanthorhynchus* Travassos, 1915.

SYNONYMS :—*Echinorhynchus* Zoega, 1776, in part.
Gigantorhynchus Hamann, 1892, in part.
Hamania Travassos, 1915.
Hamanniella Travassos, 1915.

Diagnosis.—Sexual dimorphism not well-marked. Lemnisci filiform or cylindrical, long, with numerous nuclei. Genital organs of the male situated posteriorly and occupying about a quarter of the body cavity. Testes ellipsoidal. Parasitic in mammals (marsupials and edentates) and birds.

Type species : *O. spira* (Diesing, 1851).

In place of the genus *Oligacanthorhynchus*, Travassos recognises two genera, namely, *Oligacanthorhynchus* and *Hamanniella*, which are very closely allied but, according to Travassos, may be distinguished as follows :—

Prostatic glands ellipsoidal, in pairs. Parasitic in birds..... *Oligacanthorhynchus*
Prostatic glands nail-like, condensed. Parasitic in marsupials and edentates..... *Hamanniella*

The distinction based on the shape of the prostatic glands appears to us to be difficult to make out, and is not clearly shown in at any rate one of Travassos' figures, and therefore we have included both genera in a single genus for which the name *Oligacanthorhynchus* appears to have priority.

A single species belonging to this genus was found in the collection, namely :—

Oligacanthorhynchus microcephalus (Rud., 1819).

SYNONYMS :—*Echinorhynchus microcephalus* Rud., 1819.
Hamania microcephala Travassos, 1915.
Hamanniella microcephala Travassos, 1915.

One male specimen from the intestine of *Didelphis marsupialis*. British Guiana, 1912 (Dr. Minett).

Unfortunately the proboscis is missing, and consequently a definite identification is not possible. The incomplete worm measured about 35 mm. in length. Our specimen agrees in general with Travassos' figure of the male of this species, excepting that the lemnisci are, relatively, extremely long (about 17 mm.) and extend to the testes. Our specimen is young and consequently much shorter than the fully-developed specimen figured by Travassos, the length of which is given as 150 mm. to 200 mm. ; this fact probably accounts for the apparent difference in the length of the lemnisci in the two specimens.

Genus (3) *Prosthenorchis* Travassos, 1915.

SYNONYMS :—*Oncicola* Travassos, 1916.
Pardalis Travassos, 1917, preoccupied.
Echinopardalis Travassos, 1918.

Diagnosis.—Oligacanthorhynchidae of small to medium size. Sexual dimorphism not well-marked. Body rugose. Proboscis sub-spherical, armed with five or six transverse rows of hooks. Testes situated in the middle third of the body or more anteriorly ; genital organs of the male occupying two-thirds or more of the body cavity. Ejaculatory canal very long. Parasitic in mammals and birds.

Type species : *P. spirula* (Olfers, in Rudolphi, 1819).

In place of the genus *Prosthenorchis*, Travassos recognises three genera, namely *Oncicola*, *Echinopardalis*, and *Prosthenorchis*, which are very closely allied but, according to Travassos, may be distinguished as follows :—

1. Testes small, round. Prostatic glands large, condensed, situated just behind the testes..... *Oncicola*
   Testes larger, ellipsoidal. Prostatic glands not unusually large.....2
2. Prostatic glands ovoid, in pairs..... *Echinopardalis*
   Prostatic glands ellipsoidal, not in pairs..... *Prosthenorchis*

Some of Travassos' figures, however, do not fully support these distinctions, and therefore we have included all three in a single genus for which the name *Prosthenorchis* has priority.

Two species belonging to this genus were found in the collection, namely :—

Prosthenorchis spirula (Olfers, in Rud., 1819).

SYNONYMS :—*Echinorhynchus spirula* Olfers, in Rud., 1819.
Echinorhynchus elegans Diesing, 1851.
Prosthenorchis elegans Travassos, 1915.

Nine specimens, including two males, from the intestine of monkeys ; species and locality unknown.

In Travassos' figure of the male of *P. elegans*, the worm is shown to be short and broad, the lemnisci overlap the anterior testis, the two testes strongly overlap, and the prostatic glands are compacted into a single oval mass immediately behind them. In his figure of *P. spirula*, the entire worm is shown elongated, the lemnisci, although long, extend only half-way to the anterior testis, the two testes do not overlap but are situated one behind the other in the middle third of the body, and the prostatic glands are placed single file one behind the other, forming a long cylindrical mass.

Our male specimens show characters intermediate between the two above species ; thus, the body is relatively long, the lemnisci overlap the anterior testis, the testes are slightly separated, lying one in front of the other, and the prostatic glands in one male form a compact mass as in *P. elegans*, but in the other, the anterior glands are drawn out as in *P. spirula*, whilst the posterior glands are compacted as in *P. elegans*.

For the above reasons we consider *P. elegans* is indistinguishable from *P. spirula*.

It may be noted that in our specimens the eggs were similar in shape and size to those figured by Travassos for both the above species, and that the average measurements of ten eggs were 78μ by 47μ.

Prosthenorchis pardalis (Westrumb, 1821).

SYNONYMS :—*Echinorhynchus pardalis* Westrumb, 1821.
Echinorhynchus ovatus Leidy, 1850.
Echinorhynchus campanulatus Diesing, 1851.
Echinorhynchus oncicola v. Ihering, 1902.
Oncicola oncicola Travassos, 1916.
Pardalis pardalis Travassos, 1917.
Echinopardalis pardalis Travassos, 1918.

Numerous specimens, males and females, from the intestine of *Felis pardus*. Freetown, Sierra Leone, 10.III.1923 (Professor B. Blacklock and Dr. S. Adler).

Size. The females measured from 7 mm. to 14 mm. in length, and from 1·2 mm. to 1·8 mm. in breadth ; only one of the females, viz., the longest, was gravid. The males varied in length from 8 mm. to 15 mm., and in breadth from 1·3 mm. to 1·8 mm. ; only the larger males were mature. Travassos states that *Echinopardalis pardalis* has the following measurements : female, 30 mm. to 40 mm. by 1 mm. to 2·5 mm. ; male, 30 mm. by 1 mm. to 1·5 mm. Our specimens are therefore much smaller than Travassos' specimens of *E. pardalis* and correspond more closely in length to his *Oncicola oncicola* which measures as follows :—female, 10 mm. to 13 mm. by 3 mm. to 4 mm. ; male, 9 mm. to 11 mm. by 2·5 mm. to 3 mm.

Diesing's specimens of *E. campanulatus* measured 6 mm. to 35 mm. in length, and from 2 mm. to 6 mm. in breadth.

Shape of body. Our specimens varied within fairly wide limits ; all were slightly curved, some being cylindrical and tapering at each end, whilst others were more club-shaped, the broad end being anterior. The latter included specimens which were obviously shrunken. The skin, in the majority of the specimens, was smooth and ringed, but in others it was definitely wrinkled or rugose. Our specimens possess a peculiar collar-like structure identical with that figured by Diesing for his *E. campanulatus.* Travassos states that one of the differences between *O. oncicola* and *E. pardalis* is that the former possesses a ' neck ' and the latter does not ; but at the same time he gives Diesing's *E. campanulatus* as a synonym of *E. pardalis.*

Proboscis-sheath. The muscular wall of the proboscis-sheath is very thick and, when viewed in certain positions, resembles the letter ' J.' The central nervous system is situated eccentrically, slightly posterior to the middle, and close to the end of the short limb of the muscular ' J.' The anterior ends of the muscular portion of the proboscis-sheath are connected with the proboscis by non-muscular strands.

Lemnisci. The lemnisci are very long, extending to the posterior third of the body, and often reaching nearly to the posterior extremity. In this character the specimens resemble *O. oncicola.*

Testes. These lie near the middle of the body excepting in one or two specimens in which they are situated immediately behind the proboscis-sheath. The relative position of the testes is perhaps

to some extent dependent, firstly on the body contraction, and secondly on the contraction of the muscles attached to the proboscis-sheath, which tends to move the sheath posteriorly. The testes lie one in front of the other and are about twice as long as broad; the largest testis measured 0·97 mm. by 0·46 mm. In *E. pardalis* the testes measure 2 mm. to 3 mm. in length by 0·5 mm. in breadth, whilst in *O. oncicola* they measure 0·8 mm. to 1 mm. in diameter.

The testes in our specimens thus resemble those of *O. oncicola* in length, they are intermediate between those of *E. pardalis* and *O. oncicola* in shape and appearance, whilst they resemble those of both species as regards their position.

Eggs. The eggs in our specimens averaged about 65μ by 45μ. Travassos gives the size of the egg of *E. pardalis* as 53μ to 63μ by 38μ to 42μ, and that of *O. oncicola* as 99μ by 71μ to 75μ. The eggs of our specimens thus resemble more closely those of *E. pardalis*.

It will be clear then that our specimens resemble *Oncicola oncicola* in some characters and *Echinopardalis pardalis* in others, and the facts cited above lead us to the conclusion that the two forms are identical, *Oncicola oncicola* being merely the contracted form of *Echinopardalis pardalis*.

Sub-order III. ECHINORHYNCHIDEA

Proboscis well-developed; proboscis-sheath with double walls (except in the genus *Mediorhynchus*) into which the proboscis can be retracted. Nuclei of the sub-cuticle and lemnisci relatively small and numerous, or with few large, finely dendritic nuclei. Prostatic glands not a single syncytial mass.

The order is divided into four families.

Key to the Families of the Sub-order Echinorhynchidea.

1. Proboscis long, armed with numerous hooks which are stronger on the ventral than on the dorsal aspect..... *Rhadinorhynchidae* (1)
   Proboscis armed with hooks arranged radially and symmetrically.....2
2. Proboscis sheath inserted near the middle of the proboscis-like structure, that is, the neck is armed with spines..... *Centrorhynchidae* (2)
   Proboscis sheath inserted at the base of the proboscis; neck absent or unarmed.....3
3. Anterior region of body, in males at least, clothed with cuticular spines..... *Corynosomidae* (3)
   Anterior region of body without spines.....4
4. Body moniliform..... *Moniliformidae* (4)
   Body not moniliform..... *Echinorhynchidae* (5)

Family (1) RHADINORHYNCHIDAE Travassos, 1923.

Echinorhynchidea of small to medium size. The anterior region of the body armed with scattered cuticular spines (except in the genus *Leptorhynchoides*). Proboscis long (at least twice as long as broad, and often much longer), usually bent ventrally, and armed with numerous hooks which are stronger on the ventral than on the dorsal aspect. Basal portion of proboscis often without hooks. Neck absent. Proboscis-sheath long. Central nervous system near the middle of the proboscis-sheath. Eggs with or without polar capsules. Parasitic in fish, reptiles and birds.

The family contains five genera.

KEY TO THE GENERA OF THE FAMILY RHADINORHYNCHIDAE.

1. Body not armed with spines..... *Leptorhynchoides* (2)
   Body armed with spines.....2
2. Body with ventral transverse rows of spines..... *Serrasentis* (4)
   Body without ventral transverse rows of spines.....3
3. Posterior extremity of the body in both sexes armed with a few scattered cuticular spines..... *Telosentis* (5)
   Posterior extremity unarmed.....4
4. Body covered anteriorly with scattered, powerful spines; not differentiated into two structurally distinct portions. Proboscis sub-cylindrical, hooks on dorsal and ventral aspects not differing notably in size, but more in a varied formation of their roots..... *Rhadinorhynchus* (1)

   Body with anterior region sharply differentiated structurally. Proboscis spindle-shaped, hooks on dorsal and ventral aspects differing distinctly in size..... *Arhythmorhynchus* (3)

Genus (1). *Rhadinorhynchus* Lühe, 1911.

SYNONYMS:—*Polyacanthorhynchus* Travassos, 1918.
Echinosoma Porta, 1907, preoccupied, in part.

Diagnosis.—Rhadinorhynchidae with very long, cylindrical proboscis, and very long lemnisci. Anterior portion of body not structurally differentiated from the rest. Body armed at the anterior end with large, scattered, cuticular spines, but without ventral transverse rows of body spines. Parasitic in fish and reptiles.

Type species: *R. pristis* (Rudolphi, 1802).

A single species belonging to this genus was found in the collection, namely:—

Rhadinorhynchus pristis (Rudolphi, 1802).

Synonym :—*Echinorhynchus pristis* Rudolphi, 1802.

Four males from the intestine of *Thynnus vulgaris*. Locality unknown.

Genus (2). *Leptorhynchoides* Kostylev, 1924.

Diagnosis.—Rhadinorhynchidae with very long, slightly club-shaped proboscis, and very long lemnisci. Body not armed with spines; nuclei dendritic. Parasitic in fish.

Type species: *L. plagicephalus* (Westrumb, 1821).

Genus (3). *Arhythmorhynchus* Lühe, 1911.

Diagnosis.—Rhadinorhynchidae with a long spindle-shaped proboscis; without ventral transverse rows of body spines. Lemnisci slightly longer than proboscis-sheath. Anterior region of body sharply differentiated from posterior region in structure of body wall; nuclei present in the sub-cuticle of anterior region only. Parasitic in birds.

Type species: *A. frassoni* (Molin, 1858).

Genus (4). *Serrasentis* Van Cleave, 1923.

Synonyms :—*Echinogaster* Monticelli, 1905, preoccupied.
Echinosoma Porta, 1907, preoccupied.
Lepidosoma Porta, 1907, preoccupied.

Diagnosis.—Rhadinorhynchidae with ventral transverse rows of body spines. Lemnisci very long. Parasitic in fish.

Type species: *S. socialis* (Leidy, 1851).

A single species belonging to this genus was found in the collection, namely :—

Serrasentis socialis (Leidy, 1851).

Synonyms :—*Echinorhynchus socialis* Leidy, 1851, not Leidy, 1856.
Echinorhynchus sagittifer Linton, 1889.
Echinogaster (species not stated) Monticelli, 1905.
Echinosoma sagittifer of Porta, 1907.
Echinogaster sagittifer of Lühe, 1912.

Thirty-five specimens found encysted in the body cavity of *Platycephalus fuscus* ('Flathead'). Townsville, Queensland, Australia, 12.1.1921 (Dr. P. A. Maplestone).

All the specimens were adult but immature; they varied in length from about 3 mm. to 8 mm., and the maximum breadth was about 0·6 mm. The specimens agreed in general with Linton's description of *E. sagittifer*, but the following points of difference were noted:—(1) the number of hooks on the proboscis, counted antero-posteriorly, varied from about sixteen to eighteen, and there were about twenty-four such rows; (2) the number of ventral transverse rows of body spines varied from about fourteen to sixteen. There is no neck. The lemnisci arise at the base of the proboscis, and are very long, extending a little beyond the middle of the body. Central nervous system situated about the middle of the proboscis-sheath.

Van Cleave (1918) re-described the species and later (1923) erected the genus. In his description he states that the number of spines in the ventral transverse rows varied from six to twenty-four; this presumably means on each side as stated by Linton. In our specimens the first row contained about forty-five, the number decreasing in posterior rows.

Genus (5). *Telosentis* Van Cleave, 1923.

Diagnosis.—Rhadinorhynchidae with the posterior extremity of the body adjacent to the genital orifice armed in both sexes with a few scattered cuticular spines. Genital orifice sub-terminal. Parasitic in fish.

Type species: *T. molini* Van Cleave, 1923.

Family (2). CENTRORHYNCHIDAE Van Cleave, 1916.

Echinorhynchidea of small to medium size. Proboscis-sheath inserted near the middle of the proboscis-like structure; that is to say, the neck is armed with spines. Hooks on the proboscis distinct in type from, and usually larger than, those on the neck. Central nervous system situated near the middle of the proboscis-sheath. Eggs where known without polar capsules. Parasitic in birds.

The family contains three genera.

KEY TO THE GENERA OF THE FAMILY CENTRORHYNCHIDAE.

1. With three prostatic glands..... *Centrorhynchus* (1)
   With eight prostatic glands.....2
2. Proboscis-sheath with a single wall..... *Mediorhynchus* (2)
   Proboscis-sheath with a double wall..... *Empodius* (3)

Van Cleave (1924) states 'that the names *Heteroplus* and *Mediorhynchus* have been applied to the identical generic concept,' and that, moreover, the generic name *Empodius* is a synonym of *Mediorhynchus*, and has been recognised as such by its author Travassos. As the prior name *Heteroplus* is preoccupied, the valid name for the genus becomes *Mediorhynchus*.

In suggesting this synonymy, Van Cleave has apparently disregarded one of the characteristics of his genus *Mediorhynchus*, namely, that 'the wall of the proboscis receptacle is composed of a single muscular layer instead of two layers' (a feature which is well shown in his figure accompanying his description of the type species *M. papillosus*), for in the genera *Heteroplus* and *Empodius* the proboscis-sheath has a double wall. Again, on comparing the figures given by Van Cleave of the proboscis-like structure of *M. papillosus*, the type species of the genus *Mediorhynchus*, and of *M. grandis*, which was subsequently placed by him in the genus *Heteroplus*, there is seen to be an important difference, the number of longitudinal rows of hooks on the neck being in *M. papillosus* about the same as on the proboscis proper (in this respect resembling species of the genus *Centrorhynchus*), whereas in *M. grandis* they are much more numerous.

Having regard to these two important differences we are unable to accept without further explanation Van Cleave's suggested synonymy, and we therefore recognise in this paper two genera in place of his *Mediorhynchus*.

With regard to the genus *Micracanthorhynchus* Travassos, 1917, Van Cleave maintains that it is a synonym of his *Mediorhynchus*. He bases this conclusion on a re-examination of Rudolphi's type of *E. micracanthus*, a species which Travassos states is closely related to *M. emberizae*, the type species of the genus *Micracanthorhynchus*. Van Cleave has figured the anterior extremity of *E. micracanthus*, and from this figure it appears probable that the species should be referred to the genus *Empodius*.*

Genus (1). *Centrorhynchus* Lühe, 1911.

Synonyms:—*Paradoxites* Lindemann, 1865.
Chentrosoma Monticelli, 1905, in part.

Diagnosis.—Centrorhynchidae having a proboscis-sheath with double walls. Proboscis and neck bearing approximately equal

* See Addendum to this paper, p. 182.

numbers of longitudinal rows of hooks. Prostatic glands three (Van Cleave), long and tubular.

Type species : *C. aluconis* (Müller, 1780 or 1784).

A single species belonging to this genus was found in the collection, namely :—

Centrorhynchus asturinus (Johnston, 1913).

Synonym :—*Gigantorhynchus asturinus* Johnston, 1913.

One male and one female from the intestine of the sparrow-hawk (*Accipiter cirrocephalus*). Townsville, Queensland, Northern Australia (Dr. P. A. Maplestone).

The male measured 18 mm. in length, and the maximum breadth was 0·6 mm. The female measured 25 mm. in length, and the maximum breadth was 0·8 mm. The body is slightly curved and cylindrical ; in both specimens there was a small constriction which, in the male, was situated immediately behind the testes, and in the female a little way behind the ends of the lemnisci. The cuticle is smooth. The proboscis-like structure measures 0·85 mm. by 0·25 mm. ; it is armed with numerous hooks radially arranged in about forty antero-posterior rows of about thirty hooks each. The hooks on that portion of the proboscis anterior to the insertion of the sheath are larger than the rest, and have long rectangular roots. The neck is marked off from the commencement of the body proper by a slight constriction.

Proboscis-sheath. The sheath arises a little anterior to the middle of the proboscis-like structure ; it measures about 1·3 mm. in length, and the maximum breadth is about 0·25 mm. The central nervous system lies a little posterior to the middle of the sheath.

Lemnisci. These organs extend backwards to a level a little posterior to the proboscis-sheath.

Testes. The testes are oval, lie one in front of the other, and are in apposition. They lie immediately behind the proboscis-sheath. Each testis measures about 0·9 mm. in length and 0·28 mm. in breadth.

Prostatic glands. These commence immediately behind the testes and are cylindrical and extremely long.

Female. The posterior extremity of the female is produced into a short, blunt, conical protuberance.

Eggs. These measure about 55μ by 22μ, and are without polar capsules.

Johnston's original description (1913) was based on the examination of a few specimens from *Astur novae-hollandiae* obtained in the neighbourhood of Townsville. He pointed out that the specimens were very much coiled, and we presume he had difficulty in examining them fully because he subsequently published an emended description (1918).

In addition to the two well-preserved specimens described above, we have at our disposal a few other specimens of this species from the same locality, obtained from the intestine of a white goshawk (*Astur novae-hollandiae*). These specimens were very much coiled, as were Johnston's. An examination of these coiled specimens showed clearly that they were morphologically identical with the specimens from *Accipiter cirrocephalus*, but were a little longer and narrower.

We have also examined three male and seven female specimens of the same species from the intestine of a grey goshawk (*Astur clarus*) obtained in the neighbourhood of Townsville, 3.6.1912 (Dr. P. A. Maplestone). The largest female specimen measures 60 mm. in length and 1 mm. in breadth. The terminal papilla noted above is absent, the specimen being distended with eggs.

Also one male and two females, all immature, from the intestine of a brown hawk (*Hieracidea orientalis*) obtained in the neighbourhood of Townsville, 19.6.1913 (Dr. P. A. Maplestone). The only point in these specimens calling for comment is the relative position of the testes which are situated a little in front of the middle of the worm. The prostatic glands are rudimentary. These differences are probably due to the worm being immature.

There seems to be little doubt but that all the forms examined by us are specimens of *Centrorhynchus asturinus* (Johnston, 1913). If this surmise is correct, then Johnston's description can be somewhat amplified by details observed in the better preserved specimens, especially with regard to the number and character of the hooks on the proboscis.

Genus (2). *Mediorhynchus* Van Cleave, 1916.

Diagnosis.—Centrorhynchidae having a proboscis-sheath with a single wall. Longitudinal rows of hooks on the proboscis and neck similar in number. Prostatic glands eight, rounded or pear-shaped.

Type species: *M. papillosus* Van Cleave, 1916.

Genus (3). *Empodius* Travassos, 1916.

Synonyms:—*Heteroplus* Kostylev, 1914, preoccupied.
Micracanthorhynchus Travassos, 1917.

Diagnosis.—Centrorhynchidae having a proboscis-sheath with a double wall. Proboscis and neck bearing different numbers of longitudinal rows of hooks, those on the neck being the more numerous. Prostatic glands eight, rounded or pear-shaped.

Type species: *E. empodius* Skrjabin, 1913.

A single species belonging to this genus was found in the collection, namely:—

Empodius segmentatus (Marval, 1902).

Four females from the intestine of a guinea fowl (*Numida ptilorhynchus*). Transvaal, 1907 (G. Arnold). Also three males and five females from the intestine of a guinea fowl (*Numida ptilorhynchus*). Upper Shire, Nyasaland, 1911 (Professor R. Newstead and Dr. Davey).

The males measured from 62 mm. to 74 mm. in length, and the greatest breadth was 2·3 mm.; the number of pseudo-segments varied from fifty-eight to seventy-three. The females measured from 65 mm. to 90 mm. in length, and the greatest breadth was 2·3 mm.; the number of pseudo-segments varied from sixty-three to eighty-eight. The body is tape-like and flattened laterally, and the pseudo-segments extend practically to both extremities; the body is broadest anteriorly and tapers gradually and continuously towards the posterior extremity. In the female the posterior extremity is bluntly rounded, but in the male, when the bursa is retracted, there are at the posterior extremity two conspicuous lateral folds.

Proboscis. In the majority of our specimens the proboscis is retracted and the anterior extremity of the worm is quite rounded.

In some specimens the proboscis lies slightly ventrally, whilst in others it is median. When the proboscis is completely protruded it is continuous with the anterior part of the body, from which it can only be distinguished by the presence of large hooks. In this condition it is evident that two distinct types of hooks are present on the anterior part of the worm, namely, a few large hooks situated anteriorly, on the proboscis, and a large number of small hooks situated more posteriorly, on the neck. When the proboscis is retracted, as it is in most of our specimens, the cuticle at the anterior extremity is invaginated and consequently the small hooks are more or less hidden, the number visible depending on the degree of retraction.

The proboscis is small and bluntly conical; it measures about 0·25 mm. in length, and its diameter across the base is about 0·4 mm. It is armed with about twenty antero-posterior rows each composed of four hooks; the hooks measure about 45μ to 55μ, and have large root-like processes. On the neck are at least forty antero-posterior rows each composed of about four hooks; the hooks are very delicate, slender, and decrease in size posteriorly, the anterior hooks measuring about 26μ to 40μ in length. These small hooks have no root-like processes.

Proboscis-sheath. The proboscis-sheath has double walls and arises at the base of the proboscis proper; it is slightly curved and tapers a little posteriorly. It measures about 1·2 mm. in length, and its greatest breadth is 0·4 mm. The central nervous system is situated about the middle of the sheath.

Lemnisci. These measure about 3 mm. to 4 mm. by 0·3 mm.

Testes. The position of the testes varies slightly, but in all our specimens they lie in the posterior quarter of the worm. They are separated from each other by a short interval. Each testis is an elongated oval body measuring from 2·7 mm. to 3·8 mm. in length and in greatest breadth from 0·9 to 1·1 mm.

Prostatic glands. These lie a little distance behind the testes, and consist of eight more or less elongated bodies, loosely compacted together. In one male they extended over 7 mm. of the body length, but in another over only 3·9 mm.

Eggs. The average size of ten eggs was 87μ by 50μ.

In 1902, Marval described a worm from *Numida ptilorhynchus*

to which he gave the name *Echinorhynchus segmentatus*. Of this worm he had only a single specimen, the sex of which was not determined, and the proboscis of which was missing. His description, therefore, was necessarily incomplete, but considering the facts that the worm came from the same host as our specimens, that its body was divided into a similar number of pseudo-segments, and that the eggs were alike, we have little hesitation in concluding that it was probably of the same species as our specimens, and accordingly we have adopted Marval's specific name. From our more abundant and complete specimens we have been able to supplement Marval's earlier description.

Family (3). Corynosomidae nom. nov.

Echinorhynchidea of small to rather large size. Anterior region of the body in the males, and (except perhaps in some species of *Filicollis*) in the females also, clothed with closely-set cuticular spines which extend backwards as a mantle for a variable distance. Proboscis armed with hooks arranged radially and symmetrically, i.e., without any distinction in size between those situated dorsally and those situated ventrally. Neck, when present, without spines. Eggs either with or without polar capsules. Parasitic in cetacea, birds and fish.

The family contains five genera.

Key to the Genera of the Family Corynosomidae.

1. Proboscis covered by a thick hyaline membrane beyond which the hooks protrude only a short distance. Central nervous system at anterior end of proboscis-sheath..... *Tegorhynchus* (5)

   Proboscis not covered by such a membrane. Central nervous system at, or posterior to, middle of proboscis-sheath.....2

2. Body proper dilated into a bulb anteriorly..... *Bolbosoma* (2)

   Body proper not so dilated.....3

3. Proboscis bent ventrally at an angle with the axis of the body. Spines on the anterior part of the body extending backwards much further ventrally than dorsally..... *Corynosoma* (1)

   Proboscis not bent ventrally. Spines on the anterior part of the body not extending backwards much further ventrally than dorsally.....4

4. Body sac-like, not notably thickened anteriorly. Prostatic glands irregularly egg-shaped..... *Filicollis* (4)

   Anterior region of body thickened. Prostatic glands tubular... *Polymorphus* (3)

Genus (1). *Corynosoma* Lühe, 1904.

Diagnosis.—Corynosomidae of small to medium size. Body club-shaped, the anterior end thickened but not separated from the posterior part. Spines on anterior part of body extending much further backwards ventrally than dorsally. Genital opening in the male armed with hooks. Proboscis bent ventrally, often spindle-shaped and unarmed at the base. Central nervous system near the middle of the proboscis-sheath. Lemnisci short. Eggs with polar capsules. Parasitic in birds.

Type species : *C. strumosum* (Rudolphi, 1802).

Genus (2). *Bolbosoma* Porta, 1908.

Synonym :—*Bolborhynchus* Porta, 1906, preoccupied.

Diagnosis.—Corynosomidae of rather large size. Body proper dilated anteriorly, a little behind the proboscis, into a bulb. Spines on the anterior part of the body not extending posterior to the dilation. Proboscis short, sub-cylindrical, unarmed at its base. Neck absent. Central nervous system near the middle of the proboscis-sheath. Eggs long and narrow, with polar capsules. Parasitic in cetacea.

Type species : *B. capitatus* (v. Linstow, 1880).

Genus (3). *Polymorphus* Lühe, 1911.

Diagnosis.—Corynosomidae of small size. Body thickened anteriorly, sometimes narrowed immediately behind the spine-bearing region. Spines on the anterior part of the body not extending backwards much further ventrally than dorsally. Genital opening in the male unarmed. Proboscis sub-cylindrical, often unarmed at the base. Central nervous system in the posterior third of the proboscis-sheath. Lemnisci of moderate length. Prostatic glands tubular. Eggs spindle-shaped, with polar capsules. Parasitic in birds.

Type species : *P. minutus* (Zed., 1800).

A single species belonging to this genus was found in the collection, namely :—

Polymorphus minutus (Zed., 1800).

Four females from the intestine of *Anas* sp., Egypt.

The specimens measured about 4 mm. to 6 mm. in length, and the greatest breadth was 1·3 mm.

The body is short and broad, tapering towards the posterior extremity, which is bluntly rounded. In one female the diameter of the anterior part was 1·3 mm., and that of the posterior part was 0·66 mm. Van Cleave states that in the genus *Polymorphus* the 'anterior end of the body is swollen and separated from the more attenuated posterior region by a constriction.' In our specimens this constriction, although clearly present in one specimen, was not obvious in the others, but neither is it shown in Van Cleave's figure of the male *P. obtusus*. The cuticle is smooth, but at the extreme anterior end it is closely beset with minute spines about 18 μ long; those on the ventral surface extend rather further back than those on the dorsal surface, but they do not extend beyond the anterior fifth of the worm, whilst the dorsal spines cover only about 0·4 mm. of the anterior dorsal surface.

Proboscis. The proboscis is situated somewhat ventrally and although bent slightly ventrally it is almost in line with the body. It is sub-cylindrical, slightly narrowed both anteriorly and posteriorly, and unarmed at the base. There is no neck. The length of the proboscis is about 0·5 to 0·6 mm., and its greatest breadth 0·3 mm. The proboscis is armed with numerous large hooks radially arranged and distributed in about sixteen antero-posterior rows each composed of about nine or ten hooks. The largest hooks are situated in the middle of the proboscis, measure about 70μ in length, and have long rectangular root-like processes.

Proboscis-sheath. The sheath is curved in the form of an arc; it measures about 1·3 mm. in length and its greatest breadth is about 0·2 mm. The central nervous system lies a little posterior to the middle of the proboscis-sheath.

Lemnisci. The lemnisci are slightly longer than the proboscis-sheath, and arise at the base of the proboscis.

Eggs. The eggs in the body cavity are long and narrow and vary in size and appearance according to the degree of maturity. The fully mature egg measured about 120μ by 30μ (average of 10) and polar capsules were not seen. The embryo within it is cylindrical,

about 60 μ by 17 μ, with rounded ends ; it is of a light brown colour and its surface presents a pitted or shagreen appearance. The eggs, immediately before becoming mature, show one or two polar capsules at each end, and the contained embryo is transparent. Some eggs thus resemble Lühe's figure of the egg of *P. minutus*, whilst others resemble Marval's figure of the egg of *E. anatis*.

Genus (4). *Filicollis* Lühe, 1911.

Diagnosis.—Corynosomidae of medium size. Body sac-like, anterior part not notably thickened, armed with spines anteriorly which do not extend much further backwards ventrally than dorsally. In the male the spines are well-developed, but in the gravid female they may be very small and well-nigh unrecognisable. Genital opening unarmed. Proboscis spherical or ovate ; in the female the proboscis may be bulbular and bear hooks only on its anterior extremity. Neck long and unarmed. Central nervous system in the posterior third of the proboscis-sheath. Prostatic glands irregularly egg-shaped. Eggs with or without polar capsules. Parasitic in birds.

Type species : *F. anatis* (Schrank, 1788).

Genus (5). *Tegorhynchus* Van Cleave, 1920.

Diagnosis.—Corynosomidae of small size. Posterior extremity of body unarmed ; in the female, terminating in two short, blunt papillae. Proboscis covered by a thick hyaline membrane beyond which the hooks protrude only a short distance. Central nervous system at the anterior extremity of the proboscis-sheath. Lemnisci long, about half the length of the body. Prostatic glands elongated. Parasitic in fish.

Type species : *T. brevis* Van Cleave, 1920.

Family (4). MONILIFORMIDAE Van Cleave, 1924.

Echinorhynchidea of medium to large size. Body without spines, and divided into a large number of pseudo-segments. Neck absent. Proboscis well developed, sub-cylindrical, armed with numerous hooks which are small and have only a single, posteriorly directed root. Lemnisci filiform, long, with numerous nuclei. Testes ellip-

soidal, situated quite posteriorly. Prostatic glands eight, almost spherical, compressed. Parasitic in rodents and insectivores.

The family contains only a single genus.

Genus *Moniliformis* Travassos, 1915.

SYNONYMS :—*Echinorhynchus* Zoega, 1776, in part.
Gigantorhynchus Hamann, 1892, in part.
Hormorhynchus Ward, 1917.

With the characters of the family.

Type species : *M. moniliformis* (Bremser, 1811).

Two species belonging to this genus were found in the collection, namely :—

Moniliformis moniliformis (Bremser, 1811).

SYNONYMS :—*Echinorhynchus moniliformis* Bremser, 1811.
Gigantorhynchus moniliformis of Railliet *et al.*
Hormorhynchus moniliformis of Ward, 1917.
Echinorhynchus cestodiformis von Linstow, 1904.
Gigantorhynchus cestodiformis of Porta.
Moniliformis cestodiformis of Travassos.

A very large number of specimens were examined, from rats (*Rattus rattus* and *Rattus norvegicus*), collected in Liverpool, West Africa (Freetown and Accra), South America (Manaos), and Australia (Townsville). Also one specimen from man (British Honduras), and numerous specimens from *Cricetomys gambianus* (Accra). An examination of the above specimens led us to the conclusion that they were all of the same species and we give below a general account of its characters.

The males varied in length from 5·5 mm. to 86 mm., and the females from 7 mm. to 239 mm. In worms, from a single host, the size often varied within very wide limits, some being large and fully mature, whilst others were small and incompletely developed.

Shape. In very small and immature worms the body is sub-cylindrical and decidedly broader at the anterior extremity than it is posteriorly. In fully-developed worms, however, the body is flat, tape-like and, excepting at the two extremities, marked out into a large but variable number of pseudo-segments ; the posterior end is somewhat broader than the anterior end.

Proboscis. Relatively short, cylindrical, with a broadly rounded end. Length, 0·5 mm. to 0·67 mm., greatest breadth about 0·2 mm.

Armed with twelve to sixteen (usually twelve) antero-posterior rows each composed of ten to twelve (usually eleven) hooks. The arrangement of the hooks is not quite regular. Hooks in the middle third of the proboscis about 25μ to 30μ in length; with a single root. The size, shape, and armature of the proboscis are similar in the worms regardless of their size. The proboscis was occasionally found retracted within the sheath, and frequently the entire proboscis was invaginated into the anterior extremity of the worm.

There is no neck, but the proximal end of the proboscis is devoid of hooks.

Proboscis-sheath. Large, with a double muscular wall, arising at the base of the proboscis. Length, 0·5 mm. to 1·3 mm.; greatest breadth, 0·22 mm. to 0·42 mm.

Lemnisci. Length, 2·4 mm. to 8·76 mm. Narrow, with a few large nuclei. Often very unequal. Lemnisci largest in the largest specimens.

Testes. Situated in the posterior part of the worm where they sometimes cause a slight swelling of the body; placed close together, the one anterior to the other. In the smallest immature worm examined by us they were sub-spherical and measured respectively 44μ and 63μ in diameter. In all the other specimens they were oval and usually elongated, in length varying from 201μ to 4 mm., and in greatest breadth from 120μ to 0·96 mm.; as a rule, in fully-developed worms they measured about 2 mm. to 2·5 mm. by 0·4 mm. In one specimen only a single testis was present.

Prostatic glands. Situated a little behind the posterior testis. There are, apparently, eight glands which are compacted together into a single oval mass and are usually individually indistinguishable. The mass of prostatic glands in mature worms varied in length from 0·45 mm. to 3·6 mm., and in greatest breadth from 0·25 mm. to 1·1 mm. In the smallest, immature specimens, the prostatic glands were almost unrecognisable.

Eggs. Rather variable in size and appearance. When fully mature the outer shell is slightly wrinkled and the enclosed embryo brown or dark-coloured. There are no polar capsules. In thirty eggs (ten from each of three females) measured by us, the length varied from 109μ to 137μ, average 123μ, and the greatest breadth from

57μ to 63μ, average 60·5μ. Both larger and smaller eggs were, however, seen in other worms examined. It may be noted here that the smallest female examined by us in which there were eggs of the mature form (but not fully developed), measured only 13 mm. in length. In the same host we observed very much larger females (some 47 mm. long) which were without eggs.

The species is extremely variable in size, both in a single host and in different hosts. The proboscis is, however, remarkably constant in size. There seems to be no justification for dividing up the species on account of the variations in size which it exhibits.

Van Cleave (1924) states that he has examined von Linstow's type specimen of *M. cestodiformis* and that he has discovered no points of difference between this species and *M. moniliformis*. On account of the small size of the proboscis in *Hormorhynchus clarki* Ward, 1917, we are of opinion, however, that this species is probably distinct.

Moniliformis erinacei, sp.n.

One male and one female from the small intestine of a hedgehog (*Erinaceus europaeus*). Accra, Gold Coast, West Africa (Dr. J. W. S. Macfie).

The male measured about 85 mm. in length by 1·6 mm. in maximum breadth ; the female measured about 110 mm. in length by 1·5 mm. in maximum breadth. The entire body, with the exception of the anterior and posterior extremities, is divided up into about 100 obvious pseudo-segments.

Proboscis. The proboscis measured about 0·4 to 0·5 mm. in length by about 0·2 mm. in maximum breadth. It is armed with numerous hooks arranged radially and distributed in eighteen antero-posterior rows each composed of seven to eight hooks, decreasing in size posteriorly. Each hook is short and stout, the largest measuring about 30μ in length.

A pseudo-neck is present, which, when the proboscis is partly retracted, forms a prominent ruff or frill.

Proboscis-sheath. Its length is about 0·8 mm., and its greatest breadth 0·3 mm.

Lemnisci. The lemnisci are long, cylindrical, and relatively

narrow ; they measure 7 mm. to 8 mm. in length, and in greatest breadth 0·2 mm.

Testes. The testes are situated quite at the posterior extremity of the body ; they are large oval bodies measuring about 5 mm. in length and 1·3 mm. in breadth.

Prostatic glands. These form a somewhat compact mass immediately posterior to the testes.

Eggs. These resemble those figured by von Linstow, and measure (average of 10) 92μ by 51μ.

This worm agrees closely with von Linstow's description of *Echinorhynchus cestodiformis*, excepting that in the type specimens, which were from Nigerian hedgehogs, the lemnisci measured, in length, 1·7 mm. only, whilst in the specimens from the Gold Coast they measured from 7 mm. to 8 mm. Van Cleave (1924), however, has recently examined the type specimen of *M. cestodiformis* and has stated that it does not differ in any respect from *M. moniliformis*, and, therefore, the species described above, which differs from *M. moniliformis* in several respects, such as the size and armature of the proboscis and the dimensions of the eggs, must be regarded as a new species.

Family (5). Echinorhynchidae Cobbold, 1879.

Echinorhynchidea of small to medium size. Body and neck (when present) without spines. Proboscis armed with hooks arranged radially and symmetrically. Eggs with or without polar capsules. Parasitic in mammals, birds, amphibians, and fish.

This family contains a heterogeneous group of species from which, during recent years, a number have been separated as distinct genera, leaving, however, a residue of species, not yet susceptible of more exact classification, in the original genus *Echinorhynchus*.

The genus *Plagiorhynchus* we regard as only another name for *Echinorhynchus*, the species referred to it appearing to be distinct only in the length of the lemnisci, and in that they occur in birds, characters which we do not consider to be of generic importance.

The family contains five genera.

KEY TO THE GENERA OF THE FAMILY ECHINORHYNCHIDAE.

1. With three prostatic glands..... *Prosthorhynchus* (1)
   With four prostatic glands..... *Oligoterorhynchus* (2)
   With six prostatic glands.....2
2. Neck very long, expanded at its anterior extremity into a sub-spherical bulla..... *Pomphorhynchus* (3)
   Neck short or absent, without a bulla.....3
3. Central nervous system at the posterior extremity of the proboscis-sheath..... *Acanthocephalus* (4)
   Central nervous system near the middle of the proboscis-sheath..... *Echinorhynchus* (5)

Genus (1). *Prosthorhynchus* Kostylev, 1916.

Diagnosis.—We have not been able to consult Kostylev's description of this genus, but according to Van Cleave (1923) the following appear to be its chief characteristics. Body without spines. Without giant nuclei. Proboscis very long, cylindrical or clavate, armed with hooks which are arranged radially and symmetrically. Neck short, unarmed. Proboscis-sheath sac-like, with double walls. Prostatic glands three, long and tubular. Parasitic in birds.

Type species : ?

Genus (2). *Oligoterorhynchus* Monticelli, 1914.

Diagnosis.—Echinorhynchidae of medium size. Proboscis sub-cylindrical, small, armed with numerous hooks. Base of proboscis unarmed. Neck absent. Lemnisci a little longer than the proboscis-sheath. Testes oval, situated in the middle third of the body. Prostatic glands four, long, sac-like, narrow. Parasitic in birds.

Type species : *O. campylurus* (Nitzsch, 1866).

Genus (3). *Pomphorhynchus* Monticelli, 1905.

Diagnosis.—Echinorhynchidae of small to medium size. Proboscis sub-cylindrical. Neck very long, cylindrical, excepting at its anterior extremity where in some species it expands into a sub-spherical bulla. Central nervous system at the posterior end of the proboscis-sheath. Parasitic in fish.

Type species : *P. laevis* (Zoega, 1776).

Genus (4). *Acanthocephalus* Koelreuter, 1771.

SYNONYM :—*Echinorhynchus* Zoega, 1776, in part.

Diagnosis.—Echinorhynchidae of small to large size. Proboscis short, ovate or cylindrical. Neck very short. Central nervous system at the posterior extremity of the proboscis-sheath. Parasitic in amphibians and fish.

Type species : *A. anguillae* (Müller, 1780).

A single species belonging to this genus was found in the collection, namely :—

Acanthocephalus bufonis (Shipley, 1903).

SYNONYM :—*Echinorhynchus bufonis* Shipley, 1903.

Three females and one male from the intestine of a toad. Hong Kong (Dr. Bell).

The male specimen measured 9 mm. in length, and the maximum breadth was 1·5 mm. ; the females measured from 20 mm. to 25 mm. in length, and the maximum breadth (near the anterior end) was 1·5 mm. to 1·8 mm.

Body cylindrical, slightly thickened anteriorly and tapering a little posteriorly, the posterior extremity being bluntly rounded. The body is curved, especially in the female, and the skin is smooth.

Proboscis. This organ is cylindrical and is situated asymmetrically as pointed out by Shipley ; length 0·5 mm. to 0·6 mm. ; breadth 0·3 mm. It is armed with eighteen to twenty antero-posterior rows each composed of six to eight hooks. The hooks are strongly geniculated at their base and in the middle of the proboscis measure 80μ to 90μ in length. The roots resemble those described by Lühe as present in *E. ranae*, i.e., they have no lateral wing-like expansions.

Proboscis-sheath. This measures about 1 mm. in length and 0·4 mm. in breadth, and is inserted at the base of the proboscis. Neck absent, or extremely short. The central nervous system lies at the posterior extremity of the proboscis-sheath.

Lemnisci. These are about twice as long as the proboscis-sheath and are rather broad.

Testes. These are situated at the beginning of the posterior half of the body ; they measure 0·6 mm. in length by 0·5 mm. in

breadth, and, in the single specimen examined, they lie one in front of the other and are in apposition.

Prostatic glands. These glands are elongated and extend to the posterior margin of the posterior testis.

Eggs. The eggs in the body cavity measured about 75μ by 26μ.

The specimens, therefore, agree with Shipley's description excepting as regards size. They differ from *E. ranae* in (1) the greater length of the lemnisci, and (2) the greater breadth of the eggs.

Genus (5). *Echinorhynchus* Zoega, 1776.

SYNONYM :—*Plagiorhynchus* Lühe, 1911.

Diagnosis :—Echinorhynchidae of small to large size. Proboscis long, sub-cylindrical, armed with numerous circles of alternating hooks. Hooks of almost uniform size excepting those of the few basal rows which are much reduced. Neck very short or absent. Central nervous system near the middle of the proboscis-sheath. Parasitic in mammals, birds, and fish.

Type species : *E. gadi* Zoega, 1776.

The following species found in the collection are referred to this genus :—

Echinorhynchus bazae, sp.n.

One male and two females from the intestine of a crested hawk (*Baza subcristata*). Townsville, Queensland, Northern Australia, 8.12.1913 (Dr. P. A. Maplestone).

The male measured 33 mm. in length, and the greatest breadth was 2 mm. Both females were incomplete, the fragments measuring 45 mm. and 50 mm. in length respectively, and about 2 mm. in breadth. Body rugose, without spines.

Proboscis. The proboscis is short and broad, slightly constricted about the middle, broadest in the basal half, with a rounded anterior extremity. In the male it measured 0·9 mm. by 0·64 mm., and in the females 1·2 mm. by 0·7 mm. The hooks, which extend to the base of the proboscis, are arranged radially in about thirty-eight to forty-one antero-posterior rows each composed of twelve or thirteen hooks. The hooks on the distal two-thirds are larger than the rest and have long rectangular root-like processes ; the larger (anterior) hooks measure about 90μ in length.

Proboscis-sheath. The proboscis-sheath is inserted at the base of the proboscis. There is no neck. In the male the sheath measured 1·4 mm. by 0·76 mm., and in the females 1·78 mm. by 0·7 mm. The central nervous system lies about the middle of the sheath.

Lemnisci. These are slightly more than twice the length of the proboscis-sheath.

Testes. The testes are situated just posterior to the proboscis-sheath, lie obliquely one in front of the other, and measure about 1·5 mm. by 0·66 mm.

Prostatic glands. There are, apparently, six very long cylindrical prostatic glands terminating immediately behind the posterior testis.

Eggs. These measure 78μ by 41μ ; they have no polar capsules.

Echinorhynchus bulbocaudatus, sp.n.

Very numerous specimens from a bush pheasant (*Centropus phasianus*). Townsville, Queensland, Northern Australia.

The females measured about 58 mm. in length, and in greatest breadth about 1·1 mm. The male (we had only one adult at our disposal) measured about 26 mm. in length, and the greatest breadth was 0·9 mm. The cuticle is smooth. The worms are long and cylindrical. In the female the terminal 3 mm. is oval and dilated, and the body ends in a point.

Proboscis. The shape of the proboscis varies from oval to subspherical. It is small and arises somewhat obliquely. The proboscis is separated from the body by a short neck (about 0·2 mm. long), which is devoid of hooks. When the proboscis is partly retracted, as it is in most of our specimens, the anterior portion of the body overhangs its base and the cuticle is folded so as to resemble a frill or ruff. The proboscis measures about 0·5 mm. to 0·7 mm. in length, and 0·4 mm. to 0·5 mm. in greatest breadth. It is armed with numerous hooks, arranged radially and distributed in about twenty-eight antero-posterior rows, each composed of about nine hooks. The hooks in the fourth and fifth rows are the largest and measure about 45μ in length. Each hook is provided with a conspicuous, long, rectangular root, slightly hollowed out at its posterior margin.

Proboscis-sheath. This arises a little anterior to the base of the proboscis-like structure, that is, there is a short neck which is unarmed. In the male the proboscis-sheath measured 1·46 mm.

by 0·24 mm., and in the female 1·5 mm. to 1·6 mm. by 0·27 mm. The central nervous system lies in the anterior half of the sheath.

Lemnisci. These are rather more than twice the length of the proboscis-sheath and are massive; in the male they overlap the anterior testis.

Testes. These are situated obliquely one behind the other and they overlap; they lie about 0·7 mm. behind the proboscis-sheath. Each testis measures about 1 mm. by 0·6 mm. In an immature specimen, however, the testes were well separated from each other, and were situated more posteriorly.

Prostatic glands. These are, apparently, six in number, long and tubular, extending to the posterior testis.

Eggs. These measure about 60μ by 30μ and are without polar capsules.

Echinorhynchus clavula Dujardin, 1845, *nec* Hamann.

Three females and one male from the body cavity of a sea bream (*Sparus berda*). Townsville, Queensland, Northern Australia, 8.11.1920 (Dr. P. A. Maplestone). Also two males and one female from the intestine of a 'yellow tail' (*Trachurus declivis*), Australia, 8.11.1920 (Dr. P. A. Maplestone).

Echinorhynchus gadi Zoega, 1776.

Synonym:—*Echinorhynchus acus* Rud., 1802 (according to Lühe, 1911).

Four females and one male from the intestine of a haddock. Townsville, Queensland, Northern Australia (Dr. P. A. Maplestone). Also one gravid female from the intestine of a pollack (*Gadus pollachius*). Port Erin, Isle of Man (Dr. Annett). Also very numerous males and females from a codling, North Sea, October, 1922 (Professor James Johnstone).

Lühe gives the size of the eggs as 76μ by 13μ, but in our specimens they were larger and measured 107μ by 24μ (average of 10).

Echinorhynchus truttae Schrank, 1788.

Synonym:—*Echinorhynchus fusaeformis* Zeder, 1803.

Six females and six males from a trout, 11.1.1923 (A. W. Noel Pillers, F.R.C.V.S.). The females varied in length from about 11 mm. to 19 mm. They are broadest near the anterior extremity,

the maximum breadth being 1·2 mm. The largest male measured 10 mm. in length, and had a maximum breadth of 0·86 mm.

Lühe gives the size of the egg as 100μ. to 110μ in length by 23μ to 24μ in breadth ; v. Linstow states that they measure 136μ to 140μ by 23μ to 26μ. The average of ten eggs in the body cavity of one of our females was 137μ by 26μ.

Three females and four males from the body cavity of a sea bream (*Sparus berda*), 20.9.1920.

Three females and one male from the intestine of a fish (' grunter '). Townsville, Queensland, Northern Australia, 3.10.1920 (Dr. P. A. Maplestone). In these specimens the lemnisci extended slightly beyond the extremity of the proboscis-sheath.

Genus. *Lueheia* Trav., 1919 (?).

Travassos recently (1923) described under the name *Lueheia lueheia* a species of *Acanthocephala* obtained from *Thamnophilus severus* and *T. guttatus* with the following characters :—' Body broad, thick, fusiform, having large folds, milky-white in colour, measuring about 7 mm. in the case of the male and 12 mm. in the female in length, by 1·2 to 1·8 mm. in greatest breadth ; proboscis slightly globose, not invaginable in the adult, but retractile into the extremity of the body, measuring about 0·43 to 0·52 mm. in length by 0·38 to 0·46 in greatest breadth, furnished with 22 to 24 longitudinal rows of eight or nine hooks each ; the hooks increase in size from the head to the more enlarged part ; from thence, as far as the base, they grow progressively smaller ; these hooks are comparatively strong, and of three chief types, the anterior hooks are delicate, those in the middle are very strong and U-shaped, and finally, those at the base are falcated.

Measurement of hooks :—

Specimen.	Base.	Lamina.
1	0·037 mm.	0·034 mm.
2	0·037 mm.	0·042 mm.
3	0·054 mm.	0·045 mm.
4	0·068 mm.	0·059 mm.
5	0·071 mm.	0·059 mm.
6	0·048 mm.	0·054 mm.
7	—	0·048 mm.
8	—	0·048 mm.

Neck absent; sheath of proboscis club-shaped, measuring about 0·78 to 1 mm. in length by 0·26 to 0·27 mm. in greatest breadth; lemnisci six in number, cylindrical, straight in the female, curved in the male, measuring more or less 1·9 to 2·8 mm. in length; testes ellipsoid, situated some distance from the sheath but in contact with, or partly over-lapping, the lemnisci, measuring about 0·7 by 0·3 mm.; prostatic glands in contact with the nearest testis, elongated, voluminous, measuring about 1·3 mm. in length; deferent canals showing symmetrical extensions to the level of the nearest third of the prostatic glands, and joining up at the level of the most remote third, to form a voluminous seminal vesicle, in the shape of a very thick **Y** which straightens out to form the ejaculatory canal. The ejaculatory canal and the ducts of the prostatic glands measure about 0·7 mm. in length; the copulative pouch is comparatively small; eggs bearing bacilliform nuclei and without polar capsules, measuring, in the median plane, 0·078 to 0·075 mm. in length by 0·028 to 0·31 mm. in greatest breadth; small egg-ejector 1 to 1·5 mm. long.

Habitat. Small intestine of *Thamnophilus severus* and *Th. guttatus*.

Specimens in the Oswaldo Cruz Institute, No. 1888, Angra dos Reis, Rio.

This species is very closely related to *L. inscripta* W., from which it is distinguished by a proboscis with a greater number of longitudinal rows of hooks, and a greater number of hooks in each row, the hooks themselves being also larger.

'The walls of the body appear less rugose, and in this species there is a difference in the structure of the peripheral stratum near the middle of the walls of the body, where it is clear in comparison with Westrumb's species, a difference appreciable even in specimens prepared whole. The differences in the male genital organs, which are not placed within strictly defined limits, can scarcely be observed in the very young male specimen of *L. inscripta*. It is interesting to note that while the two species exist side-by-side in the neighbourhood of Angra dos Reis, yet *inscripta* is rare and generally found as isolated specimens; the other is common and found in large numbers in every carrier.'

We have, unfortunately, been unable to obtain Travassos's

description of the genus *Lueheia* and no figures of *Lueheia lueheia* are given. Travassos apparently places the genus in the sub-family CENTRORHYNCHIDAE.

The somewhat reduced proboscis, which in the adult is not retractile within its sheath, are characters which ally the species to the OLIGACANTHORHYNCHIDAE, and especially to the genus *Oligacanthorhynchus* or the genus *Prosthenorchis*, but on the other hand the body is small, the proboscis bears numerous hooks and the worm is found in birds, characters which suggest affinities with the *Echinorhynchidea*.

The species *L. lueheia* is, however, unique in possessing six lemnisci instead of two. Until we know whether the proboscis sheath has a single or a double wall, where the sheath arises and how many prostatic glands are present, it is impossible to classify the genus satisfactorily, but in any case the presence of six lemnisci is a character sufficiently striking to identify the species ; although there is, of course, the possibility (amounting, in this case, to probability) that the number ' 6 ' occurring in the description of the lemnisci is really a misprint for ' 2.'

ADDENDUM

Whilst this paper was in page proof Travassos' supplement to the Revision of the Family Gigantorhynchidae (1924) has come to hand.

In this paper he includes the genera *Micracanthorhynchus* Travassos, 1916 ; *Empodius* Travassos, 1916 = *Heteroplus* Kostylev, 1914, n.p. ; *Mediorhynchus* Van Cleave, 1916 = *Micracanthorhynchus* Travassos, 1917, in the family *Gigantorhynchidae*, and he defines the genera *Empodius* and *Mediorhynchus* as follows :—

Empodius. Proboscis armed with four transverse series of relatively large hooks and about fourteen longitudinal series of hooks with two hooks in each series. Neck sharply differentiated and armed with hooks having simple roots. Sheath of the proboscis *not* invaginable. Eggs with concentric membranes. Intestine of birds.

Mediorhynchus. Proboscis armed with from ten to twelve

transverse series of relatively small hooks and with about twenty longitudinal series of hooks, with five or six hooks in each series. Neck well differentiated and armed with small simple hooks. Hooks of the proboscis and neck situated in the centres of papilliform projections. Sheath of proboscis slightly developed. Proboscis not invaginable. Eggs with concentric membranes. Intestine of birds.

We agree that the two genera are distinguishable, but as we have found the proboscis invaginable in *E. segmentatus* we refer them to the Sub-order **Echinorhynchidea.**

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TETRACAMPOS WEDL 1861 AS A GENUS OF THE BOTHRIOCEPHALIDAE

BY

W. N. F. WOODLAND

(Wellcome Bureau of Scientific Research, Endsleigh Gardens, London, N.W.1)

(Received for publication 15 April, 1925)

In 1913, Southwell very briefly described from the Indian Siluroids *Ophiocephalus striatus*, *Labeo rohita* and *Wallago attu* a Cestode which, from the characters of the scolex, he identified as *Ophryocotyle bengalensis*, i.e., as one of the Davaineidae. In 1924, I described in some detail the anatomy of two species of Proteocephalids also from Indian Siluroids, viz., *Wallago attu* and *Macrones seenghala*, which I provisionally named *Gangesia wallago* and *G. macrones*, and I contended that the former species was almost certainly identical with Southwell's *'Ophryocotyle' bengalensis*. Southwell (1925) admits that my contention was correct, whence it follows that the specific name of my first species, assuming the retention of the genus *Gangesia*, should read *Gangesia bengalensis* (Southwell 1913).

This brief resumé of the history of this species serves to show that external characters, and especially scolex characters, cannot always be depended upon as a guide for the correct allocation of a new species in any modern system of classification. Southwell, however, has apparently not taken this view of the matter since in the communication referred to (1925) he revives an ancient undefined and inadequately-described genus first created by Wedl in 1861, viz., *Tetracampos*, and argues, once more chiefly on the basis of scolex characters, that *Gangesia bengalensis* is a second species of this genus, and that the name *Gangesia* must, therefore, lapse. This assertion that Wedl's species *Tetracampos ciliotheca* from the Nile Siluroid *'Heterobranchus' anguillaris* (= *Clarias lazera* according to Boulenger) was a Proteocephalid is very questionable. Wedl's other species (and new genus) *Marsypocephalus rectangulus* was

undoubtedly a Proteocephalid, as I have shown in a forthcoming paper (Woodland 1925), but there is every reason to believe, with La Rue* (1914), that *Tetracampos ciliotheca* was a Bothriocephalid, and I propose to give the reasons for that belief, but before doing so, it will be as well to state the evidence offered by Southwell in favour of *Tetracampos* belonging to the Proteocephalidae. This evidence, when examined, appears to consist solely of the general statement that ' the adult cestode parasites most common in fresh-water fishes belong to the genus *Proteocephalus*,' and the very superficial resemblance of Wedl's drawing of the scolex of *Tetracampos ciliotheca* to the scolex of *Gangesia bengalensis* (!). As regards the general statement, this is of course true enough, but Southwell omits to mention the fact that Bothriocephalids are also sometimes to be found in fresh-water fishes, and that at least one, and a very well-known one, viz., *Polyonchobothrium polypteri*, is to be found in a fresh-water fish from the Nile, viz., *Polypterus bichir*. I have also recently described (Woodland 1925) a new species of *Clestobothrium*—*C. clarias*—from a Nile Siluroid, *Clarias anguillaris*. As regards Southwell's comparison of Wedl's drawing of the scolex of *Tetracampos ciliotheca* with the scolex of *Gangesia bengalensis*, I may point out that the hooks of the two scolices are very different in form, and that whereas those of *Tetracampos* are in four groups and vary in size, those of *Gangesia* form a single complete circle and are of the same size, and that Southwell's remark that ' it is impossible to decide from Wedl's figure and descriptions,' whether Wedl's four ' Lappen ' (' Jeder Lappen besteht aus einem dünnwandigen, contractilen Parenchym und ragt an der Aussenseite des Kopfes als eine platte Scheibe hervor . . . Nach vorne sind diese Hautlappen (Bothridien van Beneden) näher an einander gerückt und umkreisen eine kuppelförmig hervorragende, bewaffnete Papille.') are ' really outgrowths from the head or whether they are true acetabula ' is certainly no justification for his implied assumption that they *are* outgrowths which bear acetabula, such as exist in Proteocephalids. The foregoing constitutes the whole of the actual evidence offered by Southwell in support of his contention, though in further support of his view he has gone so far as to conclude that Wedl erred in

* *Tetracampos ciliotheca*, ' because of its ventral genital pore, ciliated embryo and two bothria, evidently belongs to the order Pseudophyllidea.' (La Rue.)

describing the genital openings as being situated on the ventral surface.

A careful examination of Wedl's figures and description affords, I think, decisive evidence that *Tetracampos ciliotheca* was a Bothrio-

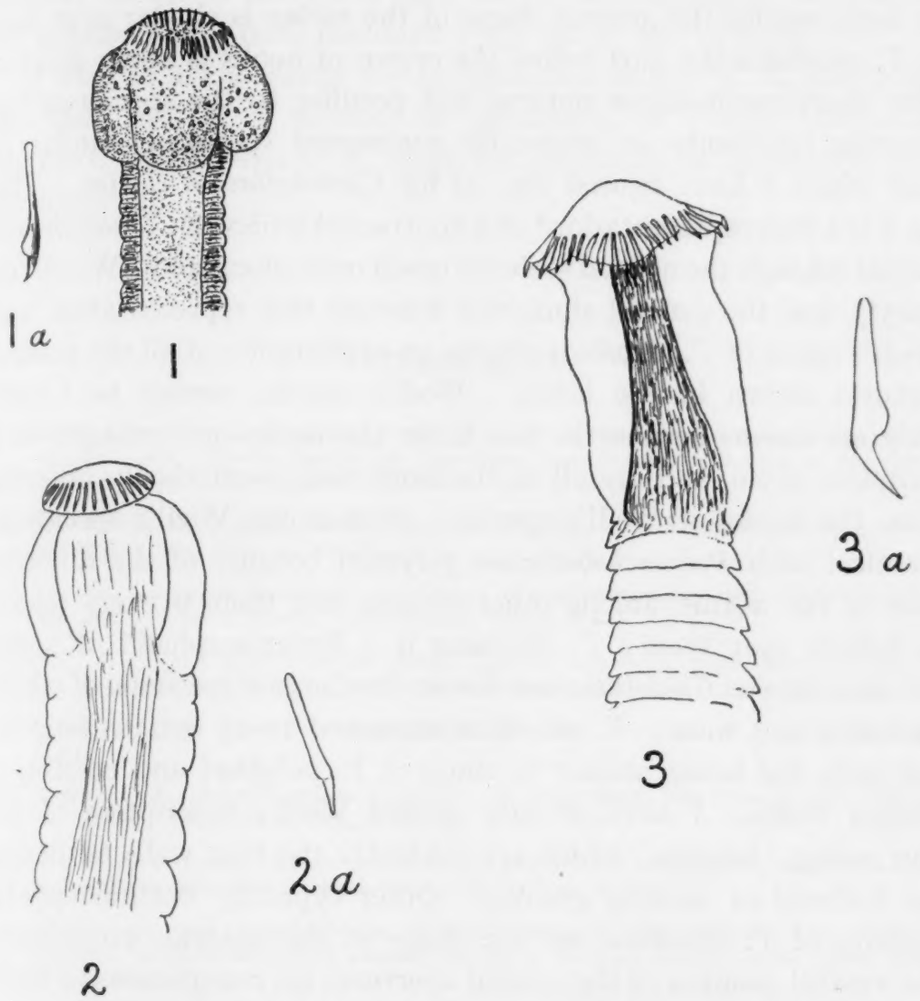

Fig. 1. Approximate copy of Wedl's figure of *Tetracampos ciliotheca*. Magnification about 100.
Fig. 1a. Approximate copy of Wedl's figure of a hook on the scolex of *T. ciliotheca*. Magnification unknown.
Fig. 2. Contracted scolex of *Clestobothrium clarias* Woodland. × 87.5.
Fig. 2a. Hook from scolex of *C. clarias*. × 395.
Fig. 3. Scolex of *Polyonchobothrium polypteri* Leydig. × 56.
Fig. 3a. Hook of scolex of *P. polypteri*. × 180.

cephalid. The hooks are very similar in form, number and arrangement to the hooks found on the crown of *Polyonchobothrium polypteri* (cf. figs. 1 and 3). In this latter species (fig. 3), as in *Tetracampos ciliotheca*, the hooks are arranged in four groups. In each group

in *T. ciliotheca* the number of hooks is usually nine (' of which the longest odd one is in the middle and the shortest pair on the outer side of each group '), while in *P. polypteri* the number varies between six and eight (Klaptocz 1906), and the hooks vary in size and in the position of the longer and shorter in each group, as in *Tetracampos*. In both species the general shape of the scolex is similar save that in *T. ciliotheca* the part below the crown of hooks is much shorter. This shortness is either natural and peculiar to the species or the drawing represents an unusually contracted specimen, similar to that which I have figured (fig. 2) for *Clestobothrium clarias*. This fig. 2 is a true representation* of a contracted scolex of *Clestobothrium clarias* (though the normal scolex is much more elongated—Woodland 1925†), and the general similarity between this representation and Wedl's figure of *T. ciliotheca* affords an explanation of all the general features shown in the latter. Wedl's species cannot be *Clestobothrium clarias* because in this latter the hooks are arranged in a complete circle, and are all of the same size, so markedly differing from the hooks of Wedl's species; neither can Wedl's species be identical with *Polyonchobothrium polypteri* because of the different sizes of the worms, among other reasons, but there is every reason to believe that Wedl's *T. ciliotheca* is a Bothriocephalid of about the same size as *Clestobothrium clarias* (my largest specimen of which measures 14·5 mm.; *T. ciliotheca* measured 10-15 mm. in length), but with the hooks similar to those of P. *polypteri* and possibly a shorter scolex. I have already quoted Wedl's description of the four scolex ' Lappen,' which are evidently the four walls bordering the bothrial or sucking grooves. Other typically Bothriocephalid features of *T. ciliotheca* are the shape of the anterior proglottids, the ventral position of the genital apertures (so conspicuous in these forms, even with an imperfect technique) and the ciliated embryophores enclosing the hexacanth embryos.

I conclude, therefore, that Southwell is mistaken in supposing that Wedl's genus *Tetracampos* has any connection with *Gangesia bengalensis* and *G. macrones*.

As regards Southwell's remarks on the systematic position of the Proteocephalidae this, of course, is a disputed subject, but I may

* As Dr. C. M. Wenyon can testify.

† This paper will provide my reason for including this species in the genus *Clestobothrium*.

say that for me the possession of lateral vitelline strands and of ventral uterine pores affords two very good reasons for relegating the family to the Tetraphyllidea, and that, with me, scolex characters count for very little, though even in this connection, Southwell appears to ignore the lobes upon which the suckers in this family are usually borne (*vide* Beddard 1913, pp. 8, 11, 12 e.g.).

I wish to acknowledge my indebtedness to Dr. H. A. Baylis for the kind gift of a number of specimens of *Polyonchobothrium polypteri*, and to Miss I. M. Bellis for assistance in connection with the literature.

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THE MEASURE OF HOOKWORM INFECTION IN COMMUNITIES

BY

ASA C. CHANDLER, Ph.D.

(*From the Hookworm Research Laboratory, endowed by Indian Jute Mills Association, Calcutta School of Tropical Medicine*)

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INTRODUCTION

In recent years the necessity for some measure of the degree, as well as the extent of hookworm infection in localities and communities, has been realised by a number of investigators, e.g., Darling (1922) and Cort (1924). It is an obvious but, nevertheless, largely ignored fact, that the percentage of individuals in whose stools eggs can be found is far from giving a true index to the severity of the hookworm situation, and yet, until very recently, this percentage has been accepted as the standard of measurement. The fallacy of this standard is, perhaps, nowhere more evident than in Bengal where, in spite of the fact that 70 per cent. or more of the population are infected, the individual infections are, on the whole, so light as to make hookworm disease in this province a comparatively unimportant problem. A correct estimate of the need for hookworm work and the judicious allocation of funds available for hookworm campaigns, as well as the strategy of campaigns, should depend on the degree as well as the prevalence of the disease. The relative value of different control measures and of different methods of treatment, also, can be correctly judged only by a consideration of the reduction in amount as well as in the incidence of infection. Hill (1923), for instance, showed that in certain areas in Porto Rico an intensive campaign reduced the percentage of infection from 87·2 to 34·1, but it reduced the egg output, which was used as a measure of the amount of infection, by 92·4 per cent.

METHODS OF MEASURING INTENSITY OF INFECTION

They may be classed as follows: (1) effects on host, (2) worm counts after anthelminthic treatment, and (3) estimation of the egg ouptut in the faeces.

Clinical symptoms, haemoglobin percentage and eosinophilia are the principal factors used in measuring effects on the host. All workers agree that the estimation of the amount of hookworm disease on the basis of clinical symptoms is difficult and complicated by differences in individual resistance, age, conditions of life, and concurrent disease; by the personal element in the classification of symptoms and severity of cases; and by the difficulty in making anything more than a very rough classification into light, moderate and severe cases. Such a clinical classification is of value in giving supplementary data as to the effects of the disease under local conditions, and in demonstrating individual and racial resistance, but it is of very little value, *per se*, as an indication of the degree of hookworm infection in a community. One might as well attempt to determine elevation on a mountain by reference to the permanent snow line, without consideration of other circumstances.

The haemoglobin content of the blood, as a measure of the degree of hookworm infection, is of little or no value in individual cases, although some authors, e.g., Darling, Barber and Hacker (1920), maintain that when sufficiently large numbers are averaged the amount of anaemia is proportional to the number of worms. Darling (1922) and Sawyer and Sweet (1922) have suggested definite ratios between the number of worms harboured and the percentage loss of haemoglobin. The haemoglobin content, however, is affected by so many factors such as sex, work, age, malnutrition, and such blood diseases as malaria, kala-azar, etc., that it can be used as a measure of hookworm infection only within wide limits. The process of elimination of other causes of anaemia is long and tedious, and in light cases there is usually no measurable drop in haemoglobin content. In a study of 100 individuals in the Alipore Central Jail, Calcutta, 67 of whom were infected with hookworm, but only six of whom had more than 1,000 eggs per gram of faeces, no difference in haemoglobin percentage between the infected and non-infected individuals could be found. The average for the uninfected ones,

according to the Tallquist scale, was 82·3, and for the infected ones 83. Two uninfected and only one infected case fell to 60 per cent., whereas one infected and one uninfected case reached 95 per cent. Darling (1922) shows that, in especially selected homogeneous groups, it requires fewer worms to cause a given loss of haemoglobin in a woman than in a man, and still fewer in a child, and also that a given number of *Ancylostoma duodenale* produces more anaemia than a similar number of *Necator americanus*, a fact which is now quite generally recognised. It is very probable that, as the number of worms increases, the haemoglobin content decreases at an accelerating rate, since it would become increasingly difficult for the patient to make good the loss produced by the worms.

Eosinophilia, as an indication of hookworm disease, is open to much the same criticisms as is the estimation of haemoglobin, since hookworm is only one of many causes of this condition. Practically all helminth infections produce more or less eosinophilia. Moreover, McVail (1922) observes that the eosinophilia in ankylostomiasis is not proportional to the number of worms present, even in uncomplicated cases, and he shows that kala-azar, and to a less extent malaria, is a powerful factor in reducing the eosinophilia due to helminthiasis.

The counting of worms passed after anthelmintic treatment, as a method of estimating the degree of infection in a community, is of unquestionable value when it can be properly carried out, but the difficulties involved are in most instances practically insurmountable. Only a small number of persons, and these especially selected ones, who can be relied upon to save all stools, can be examined in this way at a reasonable cost. The method requires a trained personnel, and cannot be left to subordinates; carelessness on the part of patients or laboratory staff, the partial failure of anthelmintics, and the loss of worms by maceration are all factors which interfere with the accuracy of the results. Even with every precaution which we have found practicable in the case of our hospital patients, we have not infrequently found lightly-infected cases to become microscopically cured after treatment, without finding any worms in the stools. It has usually been assumed that the washing of stools for 48 to 72 hours after treatment is sufficient to recover all of the worms passed, but even when a saline purge is given we have found

worms in stools as late as the sixth day after treatment, and quite frequently on the fourth day. Occasionally the stools have been negative on the second or third day, and again contained worms on the third or fourth day. For these reasons it is obvious that, however desirable a method for estimating degree of infection the worm count may be from a theoretical standpoint, it is certainly not in most instances practicable, and is always expensive.

The estimation of degree of infection by the egg output in the stools has very distinct advantages in the way of simplicity and practicability, providing that the egg output actually indicates the amount of infection. Even if this should prove to be true only to a limited extent and only when considerable numbers of individuals are averaged together, the knowledge of the number of eggs being deposited on soil, as Payne, Cort and Riley (1923) and Hill (1923) have pointed out, is, in itself, an important bit of information from the point of view of the spread of the disease. It is the egg output, and not the number of worms harboured or the clinical symptoms, which measures the public health menace.

ESTIMATION OF EGG OUTPUT

A number of different methods of estimating the actual or relative numbers of eggs in faeces have been utilised by different workers. Most of the methods of microscopic diagnosis can be used to give rough quantitative as well as qualitative information, but few of them are well adapted to give accurate information on this point. One of the first exact methods was Lane's (1918) 'standardising count,' which is Howard's centrifugal concentration technique reduced to accuracy of measurement, but this was not used for determining intensity of infection in groups or communities of people. The method devised by Stoll (1923a) is the only one which has been utilised in this way on a large scale. It is very simple, consisting merely of accurate dilution of a weighed sample of faeces in a decinormal NaOH solution, to clarify the fatty constituents of the faeces, and the accurate counting of a carefully-measured sample of the dilution. Lane (1924) has suggested the use of his direct centrifugal flotation method for this purpose, but, excellent as it may be for diagnosis, if the necessary apparatus is available,

it does not seem to me to be well adapted for quantitative work since, except where less than 500 eggs per gram are involved, the difficulty and tediousness of counting the great number of eggs thrown on the slide would counterbalance the advantage in reduced area of examination. It would be necessary, if numerous eggs were found, to repeat the process, using a much smaller quantity of stool, which would involve both time and inaccuracy due to the difficulty of measuring, say, 0·1 c.c. of stool.

The favourable results obtained by the use of Stoll's egg-counting method in Porto Rico led to a trial of it, with a few modifications, in Bengal. We have modified Stoll's method (1) by diluting the 3-gram sample of faeces to 90 c.c. instead of 45 c.c.; (2) by counting the eggs in a 0·3 c.c. sample of the dilution instead of 0·15 c.c.; and (3) by examining the preparation uncovered. There are several advantages in these modifications. In searching for eggs on an area of about two square inches on an uncovered slide, marked off by means of a glass pencil, it was found that 0·3 c.c. of the faecal suspension was necessary, under tropical conditions, to prevent the preparation from partial drying before the examination was complete. In order to get a sufficiently clear field for examination of ordinary stools with this quantity of the suspension, a dilution of 1 to 30 instead of 1 to 15 was necessary. The greatest advantage of using the larger amount of fluid and examining the preparation uncovered lay in the ability lightly to blow aside the flocculent masses of débris which often tend to hide the eggs, by gently puffing on the slide while the examination is actually in progress. Camouflage of eggs by débris is the most important source of error in all the techniques in which accurate measurements of material are made. Lane (1923, 1924) gives convincing evidence of the loss of eggs by camouflage. Maplestone (1924), in testing Stoll's method, nearly always obtained higher counts per gram when the faeces were further diluted before the egg count was made, obviously due to overlooking of eggs as a result of concealment in the more concentrated samples. By using a suspension in decinormal NaOH on an open slide with 0·3 c.c. of fluid spread over an area of 2 square inches, it is ordinarily possible, by gently blowing on the slide while making the examination, to see practically 100 per cent. of the surface of the slide with sufficient clearness to render the eggs easily visible. The eggs are heavier than

the flocculent material which makes up the great bulk of the débris on the slide, and, therefore, rest on the slide and remain visible as the overlying material is puffed aside. Furthermore, one can almost instantaneously determine whether or not an object which resembles an egg is such, since its position can be slightly changed or it can be rolled over by the same gentle puffing process. In very concentrated formed stools, we sometimes find it necessary to divide the 0·3 c.c. sample on two slides and dilute them further.

There are a few possible sources of error in this method which may be briefly commented on. In the first place, the selection of a 3-gram sample of faeces should, when possible, be made from an entire stirred stool, since the number of eggs contained in different parts is not always the same. Making duplicate counts on two samples from different parts of a single stool, the widest differences we obtained were counts of 700 and 900 eggs per gram on one sample and 1,600 and 1,800 on the other. After stirring this stool, examination of a third sample gave two counts of 1,200. In field work it is usually not practicable to get entire stools, and counts must be made on the samples submitted. As will be subsequently shown, however, the error arising from this, in individual cases, is neutralised when 50 or 100 samples are averaged.

We have tested a number of diluting fluids but found that the decinormal NaOH solution gave clear fields and more readily visible eggs than any other fluid. Addition to the NaOH of 1·5 per cent. NaCl had the effect of causing the faecal débris to clump together into large light flocculent masses which could be blown about, leaving a beautifully clear background on which the eggs showed up with striking clearness, but the occasional entanglement of eggs in these masses reduced its accuracy.

The thorough mixing of the samples in homogeneous suspensions is sometimes slow and difficult, and it is easy to overlook small masses of faeces which have failed to disintegrate. Unless carefully watched, this is one of the most fruitful sources of error. When available, a mechanical shaker is of great advantage. Settling of eggs in the diluting fluid must also be carefully guarded against; the stopper of the flask should be removed and the sample withdrawn immediately after a thorough and vigorous shaking. Even a few seconds' delay entails inaccuracy. We have found that the samples

can be withdrawn more quickly and accurately into rubber-bulb pipettes of drawn glass tubing marked at the 0·3 c.c. level, than into bacteriological pipettes. Only the required amount of fluid is sucked into the pipette, and all of it expelled on to the slide.

The NaOH solution does not appreciably change the appearance of the eggs of hookworms, *Trichuris*, *Hymenolepis nana*, or *H. diminuta*, but Ascaris eggs have the rough albuminous coat more or less completely dissolved off and thus often look quite different from the normal eggs, especially in the case of unfertilised ones. Taenia eggs undergo a peculiar change in that the embryophore swells to a diameter of from 50 to 60μ, leaving a much-enlarged clear space between it and the embryo ; the latter shrinks somewhat and assumes a characteristic elongated form.

Recounts of the same slide, duplicate counts from the same suspension, and counts on higher dilutions have shown that camouflage of eggs is practically done away with by the method here described. Lane warns against the loss of eggs held on the surface of a film too deep to be in one optical plane and in which only the bottom is searched. Apparently this rarely happens in a decinormal NaOH solution since I have several times gone over the surface of a slide containing several hundred eggs without finding a single egg. The entanglement of eggs in flocculent masses occasionally occurs, though much more frequently with Ascaris than with hookworm eggs. It usually takes a little time for the débris on the slide to clump, a process which takes place much more extensively in some stools than in others ; consequently, it only rarely happens that the eggs do not have time to settle. The blowing process also aids in liberating them. There is no doubt but that some loss of eggs does occur in these ways, but even if there were a constant loss of, say, 10 per cent. of eggs, it would be of little consequence, since what is desired is not so much an absolute knowledge of the number of eggs as a comparative measurement of the egg output.

That the method here described gives a good comparative measurement of eggs per gram of faeces is shown by the uniformity of counts which are obtained from examinations of two different samples prepared from the same stool. Where the average count on the slide is 10 or less, in about 80 per cent. of several hundred duplicate examinations, the counts were identical or within one

of each other, and, therefore, as close as possible to the average. In another 16 per cent. the counts were two numbers apart, whereas in only about 4 per cent. were the counts three numbers apart. Where the average slide count is between 10 and 100, in 35 per cent. the two counts came as near as possible to the average, in another 44 per cent. they were not over 10 per cent. from the average, whereas in only 8 per cent. were they more than 15 per cent. from the average. Where the average count exceeded 100, 87 per cent. of the duplicate counts fell within 10 per cent. of the average and none over 15 per cent. from it.

In nearly every instance in which there was any considerable discrepancy in the two counts a clumping of the eggs was observed, evidently due to their being held together by strands of mucus which had not been broken up in the shaking, in spite of an apparently homogeneous suspension. This clumping was also observed by Davis (1924), but with our technique we have only rarely obtained as irregular duplicate counts as Davis records in many of his cases; undoubtedly he was dealing with mucous stools.

In a series of about 600 faecal samples received from the Alipore Central Jail, counts have been made on two different slides prepared from a single suspension made from samples collected in quarter-ounce faeces-tins. The results which have been obtained from these counts compare very closely with those obtained from examinations of two separately prepared suspensions. This indicates that the differences in the counts are due not to variations in different parts of a stirred stool, but to errors in the counting technique. It appears, therefore, that a single suspension made from a stirred stool gives a sufficiently fair sample of the entire stool.

RELIABILITY OF EGG COUNTS AS AN INDICATION OF DEGREE OF INFECTION

It is now important to know the amount of variation which occurs in the eggs per gram of faeces in individuals according to the consistency of the stool, and from day to day. To get some light on this we studied the egg content of the stools of 36 hospital patients on from 3 to 22 different days, and made duplicate egg counts on separately prepared suspensions from 194 stools. By classifying the

stools as liquid, mushy, semi-formed and formed, and comparing the egg counts of these several groups in the case of each individual, it soon became apparent that, roughly speaking, the formed stools contained twice as many eggs and the liquid stools half as many or less, as the mushy stools. This compares fairly closely with Stoll's findings in Porto Rico (1923b). It was evident, therefore, that if intensity of infection were to be measured by egg counts, the factor of consistency would have to be considered.

Since, in India, mushy stools are normal and formed ones are rare, we accept the count on mushy ones as normal and correct the counts on formed and liquid stools by dividing or multiplying by 2. Such counts we refer to as ' corrected counts.' In a paper which has recently come to hand, Stoll (1924) arrives at exactly similar conclusions, except that he accepts formed stools as normal and multiplies the counts on mushy and liquid stools by 2 and 4 respectively, to bring them to ' basis of formed stool.'

Our counts on these preliminary 36 patients showed, however, that even when the consistency of the stool does not vary, there is a surprising variation in the egg output per gram of faeces on different days. In case 22, for instance, considering only the mushy stools, there was a maximum variation from 250 to 1,100 eggs per gram, in case 29 from 500 to 1,250, in case 32 from 250 to 1,000, and in case 35 from 50 to 350. These are the most extreme cases ; in most instances, if the consistency of the stool is taken into consideration, the variation is much less. There appears to be a much more marked tendency to vary in some individuals than in others. Stoll (1924), in a study of the egg output of two individuals, for 15 and 40 days respectively, found a similar day-to-day variation. In one of his cases the mushy stools varied from 1,000 to 2,600 and in another from 430 to 800, whereas the formed stools in the latter case varied from 400 to 1,330.

An attempt was made to get 24-hour samples of stools and to calculate the total daily egg output for 24 hours by means of the egg count and stool weight, since it seemed probable that the amount of the stool would to some extent counterbalance the variations in eggs per gram. Our results, however, failed to show any such counterbalancing tendency, since it just as frequently happened that a low egg count was accompanied by a small 24-hour output of stool

as the reverse, thus giving a greater variation in the total egg output than had been found in the number of eggs per gram. Stoll's (1924) tables show a similar lack of correlation. The most obvious reason for this appears to be that the extent to which the bowels are emptied on each day varies, even if the habits are fairly regular. In most of my cases the hour at which the stools are passed each day varies considerably, so it occurred to me that better results might be obtained by weighing only a single stool each day and keeping a record of the time between the last previous stool and the one examined. In this way we should know the number of hours during which the faeces and the eggs contained in them had been accumulating and could calculate from this the number of eggs produced in 24 hours. 112 stools from 23 different cases were examined in this way, but practically the same amount of variation was found in daily output as when 24-hour outputs of faeces were weighed without reference to time of stools, undoubtedly due to the same factor of completeness of evacuation of the bowels.

As Stoll has pointed out, it is only when the total daily output of eggs, calculated from eggs per gram and weight of stool, is averaged for at least three days that the coefficient of variation is reduced to a low level. For one-day examinations the egg count by itself gives less variable results than the calculated total egg output. Since, under field conditions, the collection and weighing of stools for three days on any considerable number of individuals is out of the question, for the same reasons that worm counts are impracticable, reliance must be placed on the egg counts alone, even though some inaccuracy is involved. Stoll has shown that in the two cases he examined, which were of widely different types, the total egg outputs showed a relation of 5·4 : 1, whereas the average corrected egg counts per gram showed a relation of 3·3 : 1. The failure of the egg counts to show a correct relationship is, of course, due to differences in food habits and consequent daily amount of faeces in which the eggs are distributed. We believe, however, that in more or less homogeneous groups, such as tea garden coolies, mine labourers, etc., habits are sufficiently alike for the corrected egg count, if averaged for three days, to give a reasonably good index of the relative egg output of different individuals. Since the egg counts of individuals approach a level when averaged for three or four days, it is obvious that in

determining the degree of infection of a group or community by counts on 50 or 100 individuals, single egg counts are quite sufficient, since variations would automatically be blotted out in the consideration of such numbers.

To test this point a study was made of 100 prisoners in the Alipore Central Jail, with the kind co-operation of the Superintendent, Lt. H. A. Young, I.M.D. A double count was made from a single suspension on two separate occasions, about a week apart. Most of the infections found were extremely light, so that although 67 were shown to be positive for hookworm, by the Kofoid and Barber technique, only 45 positives were found by examination of two slides prepared for egg counts on the first examination, and 44 on the second. Eleven which were negative on the first examination were positive on the second, and 12 which were positive on the first were negative on the second. Of these 23 cases, 12 showed only a single egg on four slides, six more gave an average of one egg per slide on the positive examination, and the remaining five gave average counts of from 1·5 to 2·5 on the positive examination. In spite of the high percentage of these low counts, which would tend to increase the probable error in the two counts, the average number of eggs per gram of faeces on the first examination was 282 and on the second 257, a deviation of only 4·6 per cent. above and below the average of the two. This compares quite favourably with the deviations of 3·9 per cent. and 3·3 per cent. from the average, which were found in the first and second slides in the first and second examinations respectively. This justifies the conclusion that a single egg count on a fair number of individuals gives a reasonably good estimate of the average egg output of that group.

Owing to the fact that we have not found it practicable to control our hospital patients sufficiently so that the preservation of all stools passed after anthelmintic treatment could be depended upon, we can give no reliable statistics on the relationship between egg counts and worms harboured. In cases in which we have reason to believe that all the stools were saved, the number of eggs per gram per female worm usually falls between 8 and 20. In one instance, however, in which duplicate counts were made for three successive days, without finding any eggs at all, although the case was positive by flotation, four female Necators were passed. In another case which

passed 16 female Necators two eggs were found on each of two slides on one day and no eggs on duplicate examinations on two subsequent days; in this case something must have inhibited oviposition on these two days. There is likely to be less variation of this kind in the field than in a hospital, where alterations in diet, drug treatments, and concurrent disease may influence both the quantity of the stool and the oviposition of the worms.

That the correlation between egg count and worms harboured is not close in individual cases is evident from the day to day variations in the count. Mhaskar (1923) gives a table of 30 cases which purports to show that there is no correlation at all. Darling (1922) on the other hand, gives a table in which a distinct correlation is shown. Smillie (1921) and Stoll (1923b) also find a correlation. On purely theoretical grounds one is forced to the conclusion that, other things being equal, there *must* be some relationship between egg output and number of worms harboured. For instance, if a patient harbouring 10 female worms produced, on successive days, 100, 500, and 200 eggs per gram of faeces, is there any reason to doubt that if he harboured 20 female worms, other conditions being the same, he would pass on each of these days approximately twice as many hookworm eggs? It is reasonable to assume, then, that when the egg output of a large number of representative individuals is averaged together, this number gives a sufficiently accurate estimate of the degree of infection so that it can be used for comparison of different groups of individuals living under similar conditions and having similar food habits, or of the same groups before and after treatment, or for the establishment of control measures. The average eggs per gram is a less accurate guide in comparing groups living under quite different conditions and having widely different food habits, but even here, within wider limits, rough comparisons can be made. This is, however, of far less value and importance, for practical purposes, than the comparison of different groups of a single area by age, sex, occupation, etc., and the comparison of such groups at different times for the valuation of the effectiveness of control measures.

ESTIMATION OF INFECTION INDEX

Although Cort (1924) suggests the substitution, in surveys, of the egg counting method for the routine faecal examinations now generally used, and describes hypothetical cases which show its advantage, it seems to me that there is fallacy in accepting either the degree of infection as determined by worm or egg counts, or the mere percentage of incidence of infection, as an index of the amount of hookworm infection in a community, or of the benefits derived from treatment or control measures. For example, let us suppose that in two communities both living under climatic and soil conditions favourable for the propagation of hookworm, the number of eggs per gram of faeces averages exactly the same, but that the sanitary conditions and habits of the people differ. In one community the majority of the people are sanitary in habits and the hookworm infection is largely confined to a few families who are backward and careless in habits, while in the other community sanitary conditions throughout are not so good and the infection is more uniformly scattered through a high percentage of the people. In such a case it is clear that the two groups should not be placed on a par, as would be the case if only the degree of infection for the group, based on egg output, were considered ; nor should the condition of the first community be considered as far superior to that of the second as the difference in percentage of infection would probably place it. From the standpoint of the general effect on the community, the probable spread of the disease, and the sanitary conditions indicated, it is important to take into consideration the number of individuals among whom the egg output is divided. Certainly the higher the percentage of individuals who are scattering a given number of hookworm eggs daily, the greater the opportunity for the spread of the disease, and the more important it is that control measures should be inaugurated. One hundred individuals each with an output of 100 eggs per gram of faeces certainly constitute a greater menace to the community than ten individuals each with an output of 1,000 eggs per gram, or one individual with an output of 10,000 per gram, since, although the total number of eggs produced is the same in each instance, the extent to which they are scattered is largely proportional to the number of persons who are passing them, and the more they are

scattered the more opportunity there is likely to be for the larvae which develop from them to gain access to new hosts. The incidence of infection, then, rather than the degree of infection, is the correct measure of the extent to which the entire community has been, and is likely to be, exposed to the infection, whereas the degree of infection rather than the incidence of it is a rough measure of the extent to which individuals have been, and are likely to be, exposed, and of the facility with which infection can occur, under the climatic and soil conditions of the locality, when carelessness in habits permits it.

It seems to me, therefore, that both factors must be taken into consideration in order to arrive at a true hookworm infection index. To do this I have tried various ways of combining the incidence and degree of infection, as indicated by eggs per gram of faeces, to obtain a number which would give a true relative index in various actual and hypothetical cases, as judged by a common-sense consideration of all the facts involved Such an index number can, I think, be obtained by taking the square root of the product of the average eggs per gram, multiplied by the percentage of infection, or, alternatively, by taking the square root of the product of the egg counts, averaged for the infected individuals only, multiplied by the square of the percentage infected, i.e., by the equation :

$\sqrt{\frac{e.p.g.}{100} \times \%^2} = I$, where *e.p.g.* stands for average eggs per gram of the infected individuals ($\frac{e.p.g.}{100}$ being the average of the eggs counted on the slides), % the percentage infected, and *I* the resulting infection index. For example, if 50 of 100 individuals have an average of 400 eggs per gram by corrected counts, the other 50 having none, the equation would be : $\sqrt{\frac{400}{100} \times 50^2} = 100$, which is the infection index. The three hypothetical cases mentioned above of a 100 per cent. infection with 100 eggs per gram, a 10 per cent. infection with 1,000 eggs per gram, and a 1 per cent. infection with 10,000 eggs per gram, are all on a par on the basis of degree of infection for the group ; they stand in the ratio of 100 : 10 : 1 on the basis of incidence of infection ; while their infection indices work out at about 100 : 32 : 10, which seems to come much nearer their true relationships. It will be seen, however, that this method of calculation

gives correct results only if all the infections are uniform, since the average implies that the egg output is evenly divided among all the infected individuals, which is seldom the case. To get a correct estimate, therefore, the entire group of infected individuals should be broken arbitrarily into sub-groups according to the size of the egg counts, and the infection index for each sub-group separately figured and then all of them added together. For example, in a community with a 60 per cent. infection, 20 per cent. with egg counts of 100 to 500 (average 300), 20 per cent. with counts of 500 to 2,100 (average 1,000) and 20 per cent. with 2,100 to 5,100 (average 3,000), the infection index, if figured for the entire group, would be: $\sqrt{\frac{1433}{100} \times 60^2} = 228$, whereas if figured for each group separately, the infection index works out as follows: $\sqrt{\frac{300}{100} \times 20^2} + \sqrt{\frac{1000}{100} \times 20^2} + \sqrt{\frac{3000}{100} \times 20^2} = 209$. We consider as very satisfactory the grouping used by Payne, Cort and Riley (1923), according to the following numbers of eggs per gram: 1-599, 600-2,099, 2,100-5,099, 5,100-11,099, and 11,100 up.

Table I gives the infection index, as worked out on a number of actual cases, based on my own work in Bengal and on statistics given by Payne, Cort and Riley (1923), and Hill (1923), in Porto Rico. It should be noted, however, that the Jute Mill statistics are not entirely correct, since the entire percentage of infection was not determined by a concentrative method, and therefore, as the egg counts run very low, a considerable number of light infections would probably be passed over by the egg counting technique, as was shown by the Alipore Jail investigation mentioned above. It is necessary, therefore, that the egg-counting method be supplemented by a concentrative technique in order to discover the light infections which would otherwise be missed. In calculating the infection index those cases which are positive by the concentrative method only, and negative on two egg-count slides, can be calculated arbitrarily as having 25 eggs per gram.

The method we have adopted, therefore, as a routine for determining the infection index, and which we recommend for general use, is as follows :—

TABLE I.

	Number Examined	% with 1-599 e.p.g.	Average e.p.g.	% with 600-2099 e.p.g.	Average e.p.g.	% with 2100-5099 e.p.g.	Average e.p.g.	% with 5100-11099 e.p.g.	Average e.p.g.	% with 11100 or more e.p.g.	Average e.p.g.	Total % infected	Average e.p.g. for the entire group	Index of Infection
Coolies in Jute Mills and Coolie Lines	143	36†	260	18	1020	4	3100	1	5700	...	...	58†	420†	140†
Coolies in Jute Mills and Coolie Lines	48	19†	275	17	1160	2	3400	...	...	...	...	38†	310†	100†
Coolies living outside Jute Mill	98	44†	242	14	830	...	...	...	...	...	...	58†	220†	109†
Prisoners in Allipore Jail (1st exam.)	100	56**	133	9	810	1	3200	1	8500	...	...	67	272	106
Prisoners in Allipore Jail (7 days later)	100	57**	144	7	1040	2	3320	1	6450	...	...	67	286	110
Cases in Porto Rico, Area C (Payne, Cort and Riley)	92	17	300*	16	1350*	25	3600*	20	8100*	17	15000*	96	7740	630
Cases in Porto Rico Areas (before treatment) (Hill)	282	28	300*	27	1350*	17	3600*	9	8100*	6	15000*	88	2820	408
Cases in Porto Rico Areas (after treatment) (Hill)	282	25	300*	6	1350*	3	3600*	1	8100*	·35	15000*	35	215	92

* These are means instead of averages, data for the latter not being available.
† These figures are too low, since no concentration method was used to discover infections too light for detection by the egg-counting method.
** These include 23 cases detected by concentration method, but negative by egg-counting method. These infections were arbitrarily figured as having 25 e.p.g.
The figures in all cases are given to the nearest integer.

(1) Determination of the incidence of infection by a concentrative technique. In my experience the Kofoid and Barber method has given the most uniformly satisfactory results; according to tests we have made it is more accurate than the Willis method or any of the usual centrifuge methods. If the necessary equipment is at hand the published evidence in favour of Lane's direct centrifugal flotation method indicates it as the method of choice, but since our centrifuges are not adapted to this method I have not had an opportunity of trying it myself. Neither the Kofoid and Barber, nor the Willis methods are reliable for light Ascaris infections; to detect these we have found Lane's levitation method the most satisfactory.

(2) Determination of eggs per gram, by examination of all positives, by the modification of the Stoll egg-counting technique here described. By preference two slides should be examined and averaged, and the count corrected according to the consistency of the stool; if, however, 50 or 100 specimens are examined, the total averages, though not the individual counts, will be very nearly correct if only one slide is examined of specimens showing two or more eggs. Specimens found positive by the concentrative technique, but negative on two egg-count slides, may be arbitrarily calculated as having 25 eggs per gram.

(3) Determination of the infection index by the equation: $\sqrt{\frac{e.p.g.}{100}} \times \%^2 =$ Infection Index, where *e.p.g.* stands for the average eggs per gram of the infected individuals, and where the equation is separately figured for different groups showing different degrees of infection, as suggested above.

In conclusion I take pleasure in acknowledging the painstaking and reliable help given by my assistant, Dr. A. K Mukerji.

SUMMARY

1. Recent investigations have shown the necessity for the measurement of the degree of hookworm infection as well as its incidence; such a measurement is of value from the point of view of the urgency, nature and valuation of methods of treatment and control.

2. Degree of infection may be measured by clinical symptoms, haemoglobin content, eosinophilia, worm counts after treatment, or by estimation of egg output in the faeces. The first three are not reliable, and the worm counts are too difficult, impracticable and expensive for use on a large scale.

3. Estimation of egg output is simple and practical, and is of value in itself as an accurate measure of potential soil pollution whether or not it indicates accurately the number of worms harboured. A modification of Stoll's egg-counting method is described and recommended for general use. Uniformity of duplicate counts indicates that it gives a good comparative measurement of egg output.

4. The consistency of the stool must be taken into consideration in estimating the egg output from the number of eggs per gram of faeces. Counts on formed or liquid stools, where mushy stools are normal, as in India, can be corrected by dividing or multiplying by 2. Day-to-day variations in eggs per gram are considerable, but the counts approach a level when averaged for three or more days. Consideration of the quantity of the stool does not lessen the variation unless averaged for at least three days, and is, therefore, not practicable in field work. The corrected egg count alone must be relied upon, and in more or less homogeneous groups we believe that this gives a reasonably good indication of the relative degree of infection in different individuals. When averages of large groups are being considered, single egg counts of individuals are sufficient.

5. In individual cases the correlation between egg counts and number of worms harboured is not very close, but when the egg counts for a group are averaged, a fair estimate of the relative numbers of worms harboured can be obtained, especially when homogeneous groups, or the same groups at different times, are compared.

6. It is not advisable to measure hookworm infection in a community by the degree of infection alone; the incidence should also be considered, since the higher the percentage of individuals who are scattering a given number of eggs, the greater the danger to the community. The incidence of infection is a measure of the extent to which the entire community is exposed to infection; in a general way it measures sanitary conditions. The degree of

infection, on the other hand, is a measure of the facility with which infection can occur under the climatic and soil conditions of the region when carelessness in habits permits it.

7. A good infection index can be obtained only by taking both factors into consideration. This can be done by means of the equation: $\sqrt{\frac{e.p.g.}{100}} \times \%^2 =$ Infection Index, where *e.p.g.* stands for average eggs per gram of infected individuals only. This equation should be separately figured for different groups falling into certain abritrary divisions according to number of eggs per gram, and all of them added together.

8. It is recommended that the infection index in survey work be determined as follows: (1) determination of incidence of infection by a concentrative technique; (2) estimation of the degree of infection by means of egg-counts; and (3) determination of the index of infection by the equation given above.

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A NEW VARIETY OF *ANOPHELES MARSHALLI* THEOBALD FROM THE BELGIAN CONGO

BY

A. M. EVANS, M.Sc.

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Anopheles marshalli var *moucheti* var. n.

FEMALE. *Head* with upright forked scales pure white anteriorly, black posteriorly; forwardly projecting tuft of long white scales reaching well beyond the base of the clypeus. Palpi with three white bands, the proximal narrow, the two distal bands very wide, equal in length, and separated by a black ring one-quarter to one-half of their length. Antennae with white scales on the second segment,

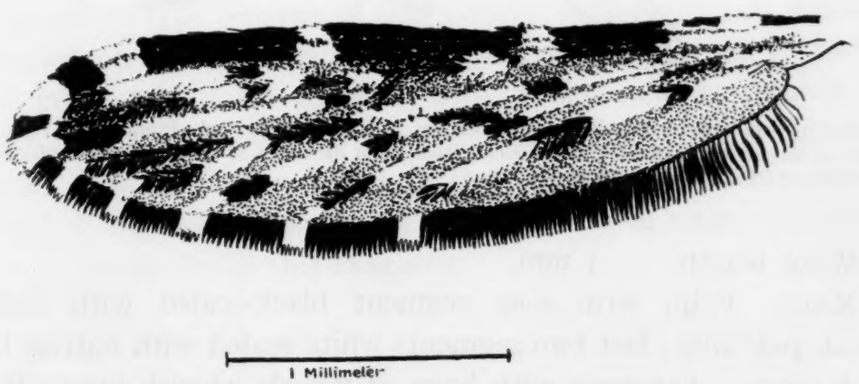

FIG. 1. *Anopheles marshalli* var. *moucheti* var. n. ♀ wing.

hairs of whorls white. *Thorax:* prothoracic lobes with blackish bristles, mesonotum with long, white, narrow, curved scales. *Abdomen* with dark integument and light brown hairs. *Wings* with white and dark scales disposed as shown in the illustration (Fig. 1). Typical plume scales from distal dark area of upper

fork of second vein (Fig. 2, A) with five striae and greatest width from one-fourth to one-fifth of the total length,* lateral squames from distal dark area of third vein (Fig. 2, B) mostly with five widely-separated striae, and greatest width one-fourth of the length. Legs black scaled; in all three pairs the tibiae and first three tarsal segments with narrow, but distinct, apical white rings. Hind legs with apical white ring also on fourth tarsal segment, mid legs with traces of pale scales apically on this segment. *Length* of white ring on hind metatarsus about equal to its greatest width, length of succeeding rings, progressively slightly shorter.

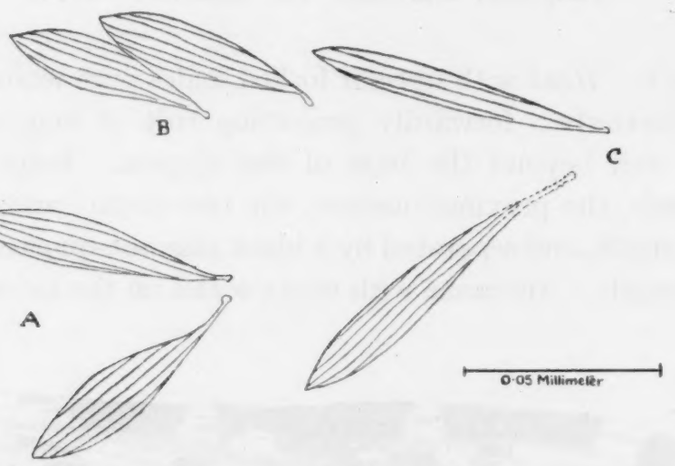

FIG. 2. *Anopheles marshalli* var. *moucheti* var. n. Wing scales. *A*—Plume scales from upper branch of second vein; *B*—Lateral squames from distal dark area of third vein; *C*—Plume scales from stem of second vein near fork.

Wing length: *c.* 3 mm.

MALE. Palpi with long segment black-scaled with narrow, apical, pale ring; last two segments white scaled with narrow basal black rings. Antennae with hairs of whorls whitish internally on proximal segments. Colouration as in the female.

The wing markings are subject to a certain amount of variation; the third pale area involving the costa and first vein may be as short on both veins as that shown on the costa in the illustration (Fig. 1), or on both veins as long as, or slightly longer than, that shown

* This description refers to scales on a wing mounted with the dorsal surface uppermost, in canada balsaam, under slight pressure.

on the first vein. The fourth vein may have the dark area on the upper branch, or the second long pale area on the stem, interrupted.

Type ♂, Buta, November, 1922, Dr. R. Mouchet; co-type ♀ ♀ (3), one from Buta, one from Bambili, and one from Api, collected in November, 1922, by Dr. R. Mouchet. Other specimens from Bambili, 5 ♀ ♀, and Buta, 1 ♂, November, 1922, Dr. R. Mouchet; Basoko, Aruwimi, 18.2.1924, Service Médicale, 1 ♂, 2 ♀ ♀; districts de l'Equator et de l'Ubangui, 18.9.1924, Dr. Trolli, 13 specimens; Kinshasa, Dr. Duren, 1922, 3 specimens.

The specimens were submitted for identification by Dr. G. Severin, of the Musée Royal d'Histoire Naturelle de Belgique, and Dr. H. Schouteden, of the Musée du Congo Belge.

Type ♂ and one co-type ♀ in the collection of the Musée Royal d'Histoire Naturelle de Belgique, the other co-type ♀ ♀ in the collection of the Liverpool School of Tropical Medicine.

The variety is named in honour of Dr. Mouchet, who has made extensive and valuable collections of Culicidae in the Belgian Congo.

This variety differs most obviously from typical *A. marshalli* Theo. in the absence of the interruption on the third large dark area of the first vein, and in the great length of the two distal white bands of the female palpi. Mr. F. W. Edwards, who very kindly compared co-type females of this variety with typical *A. marshalli*, informed me that the wing scales were shorter as well as broader than in the type form, agreeing with *A. domicolus* as regards their length, but that they were broader and denser than in this latter species. Mr. Edwards stated further that the variety resembled typical *A. marshalli* in having narrow hind tarsal rings, and differed from *A. domicolus* in this character.

THE IDENTITY OF THE RARER SCHISTOSOMES OF MAN AND THEIR INTERMEDIATE HOSTS

BY

F. G. CAWSTON, M.D., M.Sc.

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It is remarkable that at least four distinct types of Schistosome ova should occur in the urine of Natal patients, when only one type is known from the Far East and only two from Egypt. If the spindle-shaped ova were merely a variety of the ova of the *Schistosomum haematobium*, one would have expected them to occur in Egypt and if, as is thought, one type is that of *Schistosomum bovis*, one would have expected it to be more common in North Africa, in view of the heavy infestation of Sardinian cattle with this parasite and the relative immunity of South African cattle to Schistosome invasion.

In South Africa very little has been reported of the adult schistosomes and there is always an element of uncertainty where the diagnosis rests solely on the appearance of the ova that are detected in the urine of a patient.

A small ovum which is occasionally present in the urine of Natal patients has been regarded as that of *S. haematobium*, but its outline is identical with that of *S. bomfordi* Montgomery. There is certainly need for further research into the identity of those schistosomes which attack man in Africa. The subject is complicated by the difficulty that has been experienced in determining the usual intermediate host of the rarer schistosomes and because of the difficulty that therefore arises in rearing the adult parasites from the miracidia which escape from the ova in infested persons. Fig. 1 shows four different schistosome ova isolated from the urine of Natal schoolboys, as well as that of *Schistosomum japonicum* from the Far East and of two schistosomes from India.

In South Africa it is only rarely that schistosomes are found in fresh-water snails other than *Physopsis africana*. I have found schistosomes in both *Isidora globosa* Morelet and *Planorbis pfeifferi* Krauss at Laurenço Marques. Various *Isidorae* occur in South Africa ; they are very commonly infested with amphistome cercariae. *Limnaea natalensis* is the common host of *Fasciola gigantica*. *Melanoides tuberculata* Müller is the only species with an operculated shell that I have found infested with cercariae ; but J. D. F. Gilchrist has isolated cercariae from *Tomichia ventriculosa* (Sowerby) Reeve. *Ancylidae* harbour cercariae of various kinds and *Burnupia gordonensis* M. & P. is one of the commonest and largest species of this genus in South Africa.

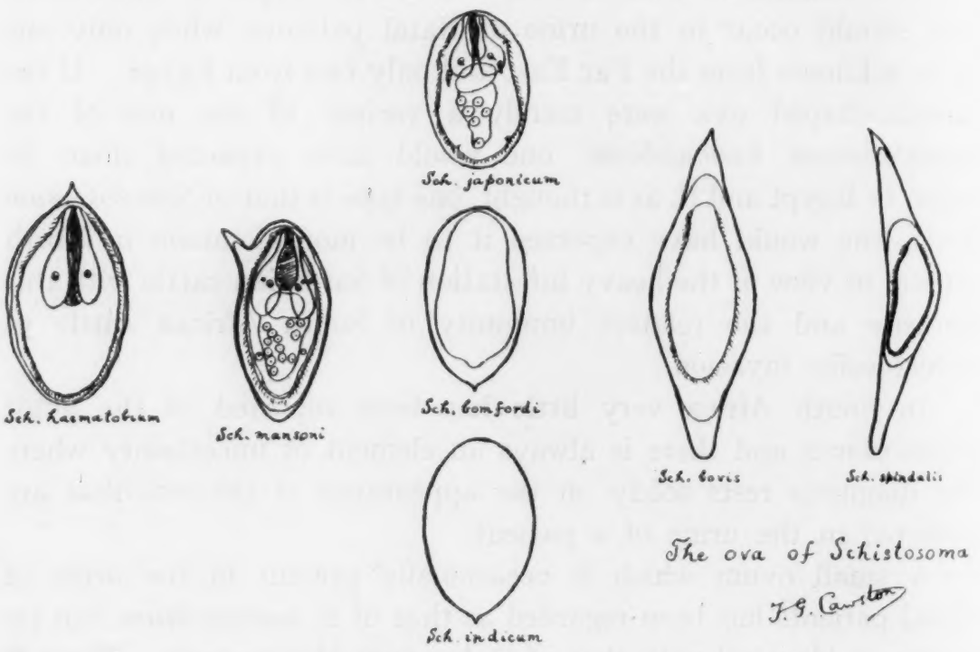

FIG. 1.

It is possible that a careful study of the radulae of intermediate hosts may assist in the determination of those species which resemble one another very closely. *Isidorae*, for instance, are notoriously variable and more than one species may occur in the same pool, each being represented by examples at various stages of growth. Although *Physae*, of which there are very few examples south of the Zambesi, might possibly be mistaken for *Isidorae* where the shell

FIG. 2. 1.—*Physopsis africana* Krauss; 2.—*Isidora globosa* Morelet; 3.—*Isidora craveni* Ancey; 4.—*Isidora tropica* Krauss; 5.—*Planorbis pfeieffri* Krauss; 6.—*Melanoides tuberculata* Müller; 7.—*Limnaea natalensis* Krauss; 8.—*Tiara coacta* Meusch; 9.—*Septaria tesselata* Lamarck; 10.—*Theodoxus natalensis* Reeve.

alone is set aside for study, there is little danger of this mistake being made where the individual teeth of the two genera are examined. Although there is a good deal of variation in the appearance of the teeth in individual examples of the same species, even when taken from the same locality and grown under apparently identical conditions, yet a careful study of the teeth reveals the fact that this variation is almost confined to the CONES which grow from the CROWN of the tooth, so that the variation is not so great as at first sight appears.

THE INCUBATION PERIOD OF BENIGN TERTIAN MALARIA

BY

G. DE M. RUDOLF

CLAYBURY MENTAL HOSPITAL

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The incubation-period of naturally-acquired benign tertian malaria is usually given as being from two to three weeks. Thus James (1920) states that the period is usually from 14 to 18 days, Stitt (1922) from 14 days, Acton cited by Castellani and Chambers (1919) 6 to 21 days, and Castellani and Chambers (1919) from 9 to 12 days. These authorities add that there are great variations in the length of the period outside the limits of the above figures. In addition, the cases of latent infection must also be remembered. In these cases several months, or even a year, may elapse since possible inoculation before the primary malarial attack develops. Often the onset is delayed until the patient undergoes some severe strain of either a mental or physical nature (James, 1920). The incubation-period of the naturally-acquired infection is thus seen to be extremely variable.

ARTIFICIALLY-INOCULATED MALARIA

(i) Mosquito-inoculations. Sir Ronald Ross (1911) gives details of cases inoculated artificially by means of infected mosquitos. The length of the incubation-period as measured by the first rise in temperature is recorded in nine instances and was found to vary from 10 to 14 to 25 days. In seven of these cases *Plasmodium vivax* was first demonstrated in from 16 to 30 days after infection.

In five general paralytics inoculated from mosquitos by Lieut.-Col. S. P. James the incubation-period varied from 11 to 16 days as regards the first definite malarial rise of temperature. In four of these cases the parasites were first found on the 17th and 18th days, three on the 17th and one on the 18th. Davidson (1925) found in a series of 23 cases that the period varied from 7 to 20 days.

In, therefore, a total of 37 cases the incubation-period, as measured by the first rise of temperature, varied from 7 to 25 days, and in 34 cases from 7 to 30 days as regards the first appearance of parasites, Davidson measuring the period by both methods.

(ii) Subcutaneous-inoculation. Most authorities give approximately corresponding lengths of time for the duration of the incubation-period when inoculation is practised subcutaneously. Thus Gerstmann (1924) states the period varies from 4 to 28 days, Scripture (1923) from 6 to 31 days, Donner (1925) from 5 to 21 days, Yorke and Macfie (1924) usually from 8 to 15 days, but with considerable variations, Worster-Drought and Beccle (1923) from 9 to 24 days and Nonne (1922) from 10 to 24 days. The table given by Pijper and Russell (1924) shows an incubation-period of from 9 to 18 days and that of McAlister (1924) from 9 to 32 days. In a series of 43 benign tertian malaria inoculations given by Grant and Silverston (1924) the first rise of temperature occurred from 1 to 18 days after inoculation, whereas parasites were first found from 6 to 22 days after. Korteweg (1924), in a series of 52 cases, found that parasites were first demonstrated in thick-films in from 5 to 21 days after inoculation.

Unfortunately, not all of the above authors state whether they define the termination of the incubation-period by the date upon which parasites were first found or by the date upon which the first increase of temperature occurred. It is, however, clear that the incubation period may be of many days' duration.

The incubation-period of a disease is that period which elapses between the admission to the body of the infecting organism and the first onset of symptoms. The latter may be subjective or objective (Gould, 1915). As the first appearance of symptoms may be objective in nature, the first day on which parasites are found in the peripheral blood-stream might be taken as the termination of the incubation-period. The disadvantage of this method, however, lies in the fact that there are so many factors to be taken into consideration when comparing the lengths of the incubation-period in different patients. At the commencement of an attack of malaria the parasites are usually comparatively few in number. In this case the day upon which the plasmodia are first found will depend upon : (*a*) whether thick or thin blood-films are used, (*b*) whether the whole or only a part of the film is examined and, if a part, which part (see below) ; and (*c*) the length of time each film is studied. In certain instances a fourth factor must be added : (*d*) the previous experience of the observer. If it were possible to

adopt general standard conditions, the method of measuring the incubation-period by the first appearance of the parasites would be of value.

With regard to the subjective symptoms it is clear that the length of the incubation-period cannot be determined by observing the occurrence of the first rigor, for many general paralytics inoculated with malaria do not shiver at all during their febrile treatment. The same applies to a subjective sensation of coldness, to sweating and to general enlargement of the spleen. The temperature, however, is raised during the initial malarial paroxysms, although, of course, parasites may be present during a relapse without fever occurring. But when the length of the incubation-period is under discussion malarial relapses do not enter into the subject. It should, however, be added that occasionally patients who have suffered from malaria previously may not develop febrile paroxysms but may show parasites for a few days. One such instance has occurred at Claybury Mental Hospital. In these cases, which are very rare, the method of recording the incubation-period by the first rise in temperature is not suitable. Now, non-inoculated general paralytics are subject, from time to time, to variations in temperature (Rudolf, 1925), and therefore an isolated elevation, or a succession of elevations, of temperature not immediately followed by typical malarial paroxysms or other definite signs of active malarial infection must not be taken as the termination of the incubation-period. As described by Korteweg (1924) and Rudolf (1924), some patients commence the attack of malaria by showing an irregularly moderately high temperature sometimes persisting for days, others by showing a series of elevations becoming progressively higher, and still others by a sudden very high elevation following a low, perhaps subnormal, temperature. Clearly, an initial rise of temperature to perhaps 100° F. in one case cannot be taken as the equivalent of a primary elevation to 105° F. in another case. To obviate this difficulty it is suggested that two rises of temperature be recorded to show the commencement of the malarial fever,—(*a*) the first elevation to 101° F. or over, and (*b*) the first to 103° F. or over. By adopting this method it is possible to tell at a glance whether a patient commenced his paroxysms suddenly or gradually. It will be observed that in this method increases of temperature under 101° F.

are not included. Now it is, of course, possible for a malarial paroxysm to show a rise of temperature of less than 101° F., but such a small rise of temperature would be extremely difficult to differentiate from an elevation accompanying the general paralysis. Rises of temperature above 101° F. are less common in untreated general paralytics.

In this connection the method adopted for recording the patient's temperature is important. From the time of inoculation the temperature should be recorded at least every four hours. Whenever it rises above normal it should be taken at least every hour, or even every ten minutes. The temperature varies so considerably within a short time that unless it is recorded very frequently the height of the fever might be missed.

The following table shows the length of the incubation-period as measured by the onset of the fever in the first 50 cases of general paralysis inoculated with *Plasmodium vivax* at Claybury Mental Hospital.

TABLE I

First rise of temp. occurring	101° F. to 102.9° F.		103° F. or over	
	No. of cases	Per cent.	No. of cases	Per cent.
Up to 10 days	23	46	19	38
From 11 to 20 days	22	44	23	46
From 21 to 30 days	5	10	8	16

The above table shows that the greater number of cases show an incubation-period of less than twenty-one days.

With regard to the first appearance of parasites, Tables IV and V show the number of days after inoculation when parasites were first found. Table IV is adapted from Korteweg (1924). This observer used the thick-film method. Table V shows the first days on which parasites were found in cases treated at Claybury Mental Hospital. Korteweg does not state that he searched the thick-films for a definite length of time and similarly the thin-films of the Claybury series were not searched during a standard time.

TABLE II. Occurrence of first rise of temperature to 101° F.

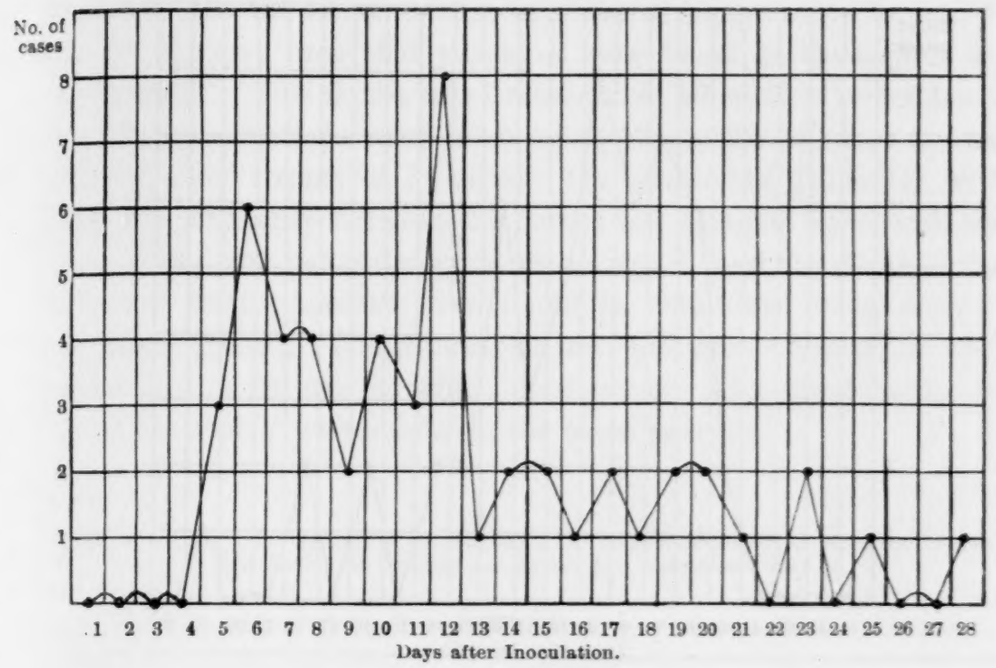


TABLE III. Occurrence of first rise of temperature to 103° F.

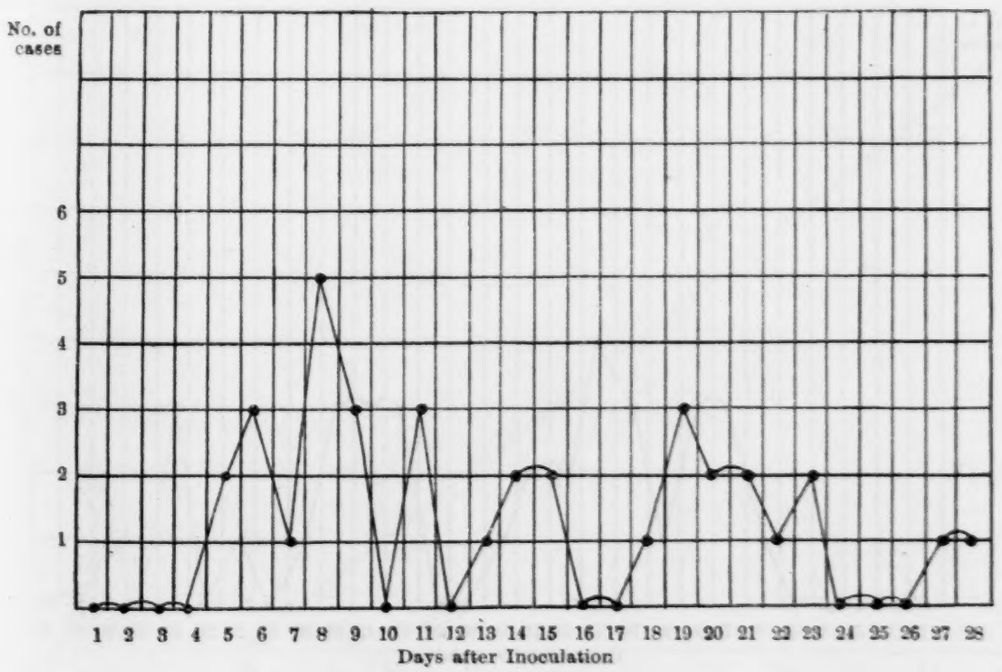


TABLE IV. First appearance of parasites in thick-films (adapted from Korteweg).

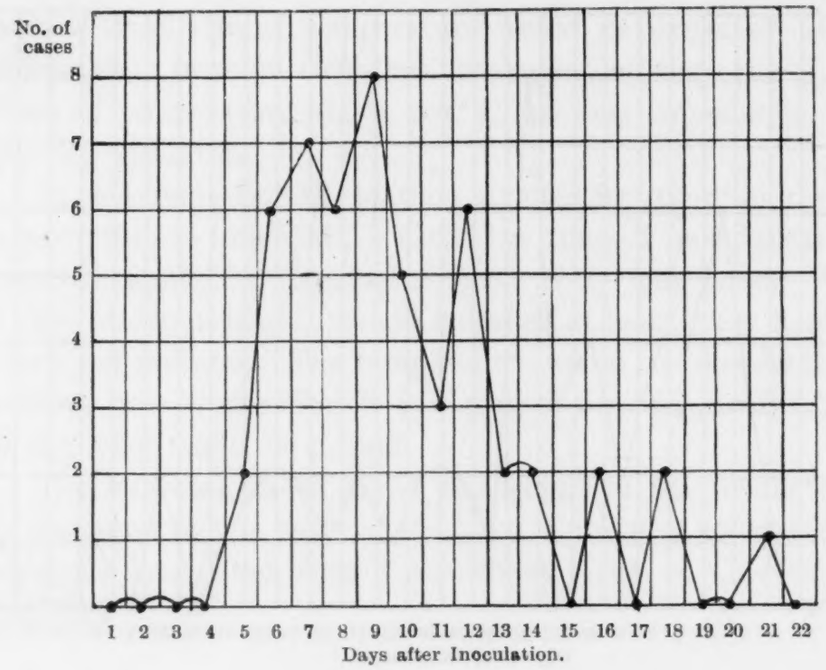
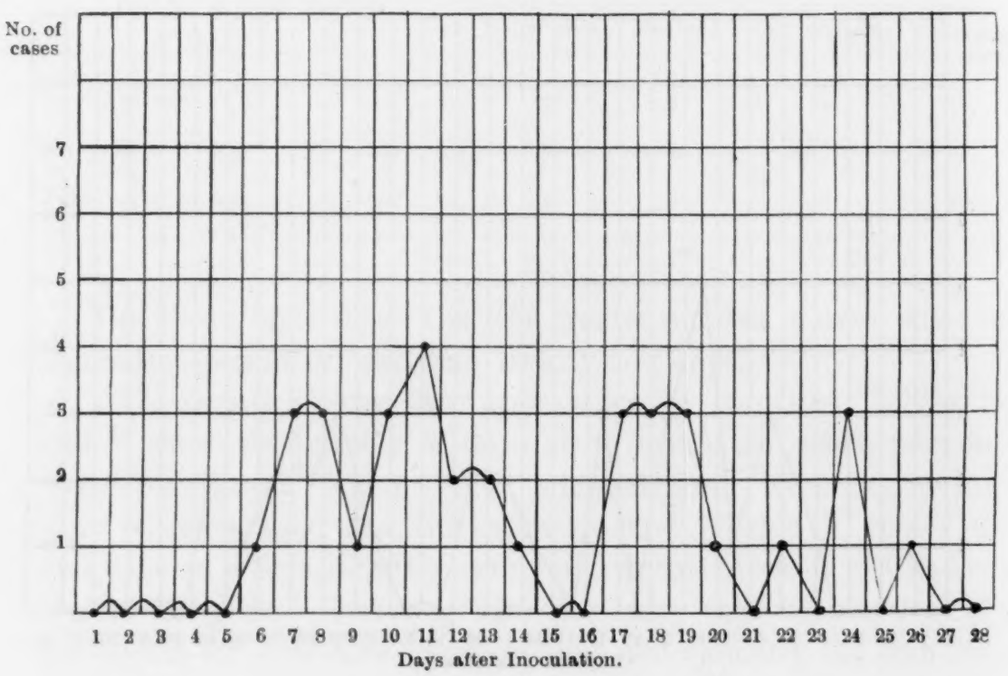


TABLE V. First appearance of parasites in thin-films.



On comparing Tables IV and V with Tables II and III it will be observed that the curves of the first rise of temperature to 101° F. and of the first time that parasites were found in thick-films are very similar. This is the more remarkable when it is remembered that the observations were made upon two series of cases treated with different strains of *P. vivax*. On comparing Table III with Table V a similarity between the curves will be again seen, both the curve of the first rise of temperature to 103° F. and the curve of the first time that parasites were found in thin-films being divided into three groups. The groups do not, however, correspond with regard to the periods in which they occur. The observations in Tables III and V were made on the same patients.

TABLES VI AND VII. Graphs of the lengths of the incubation-periods as measured by the first finding of parasites and by the first temperature-rises.

TABLE VI.

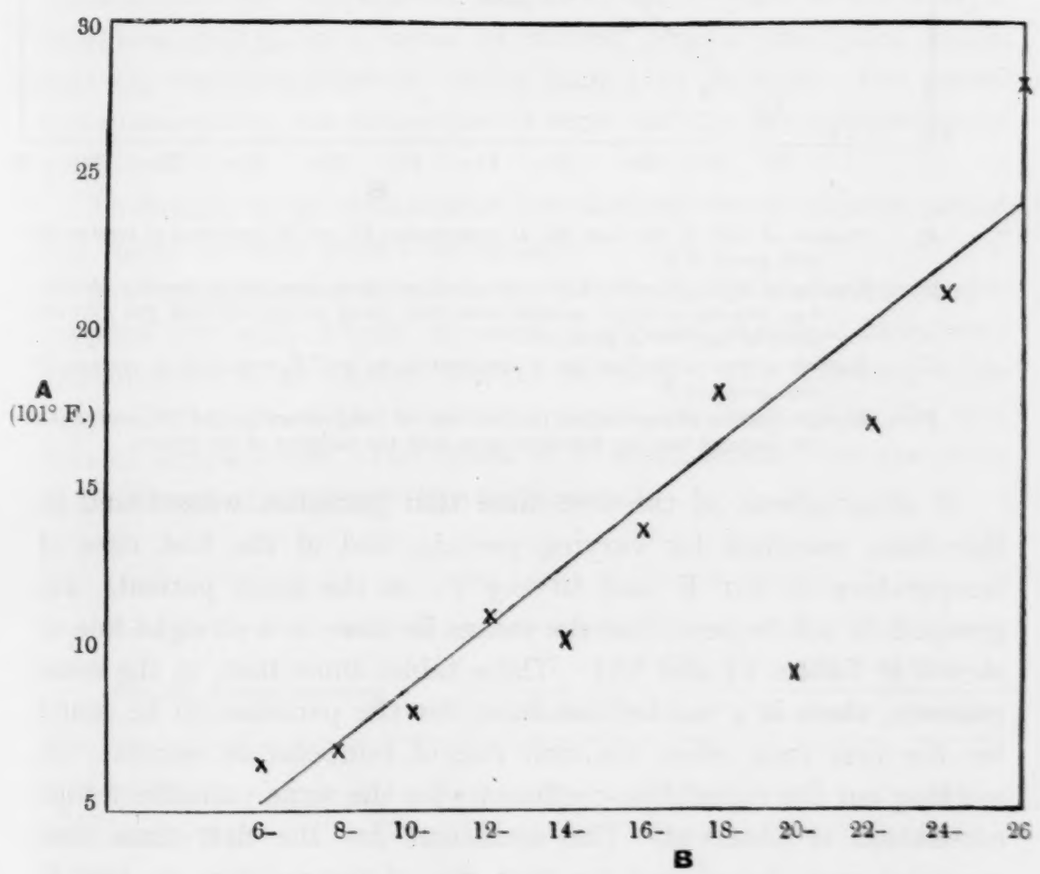
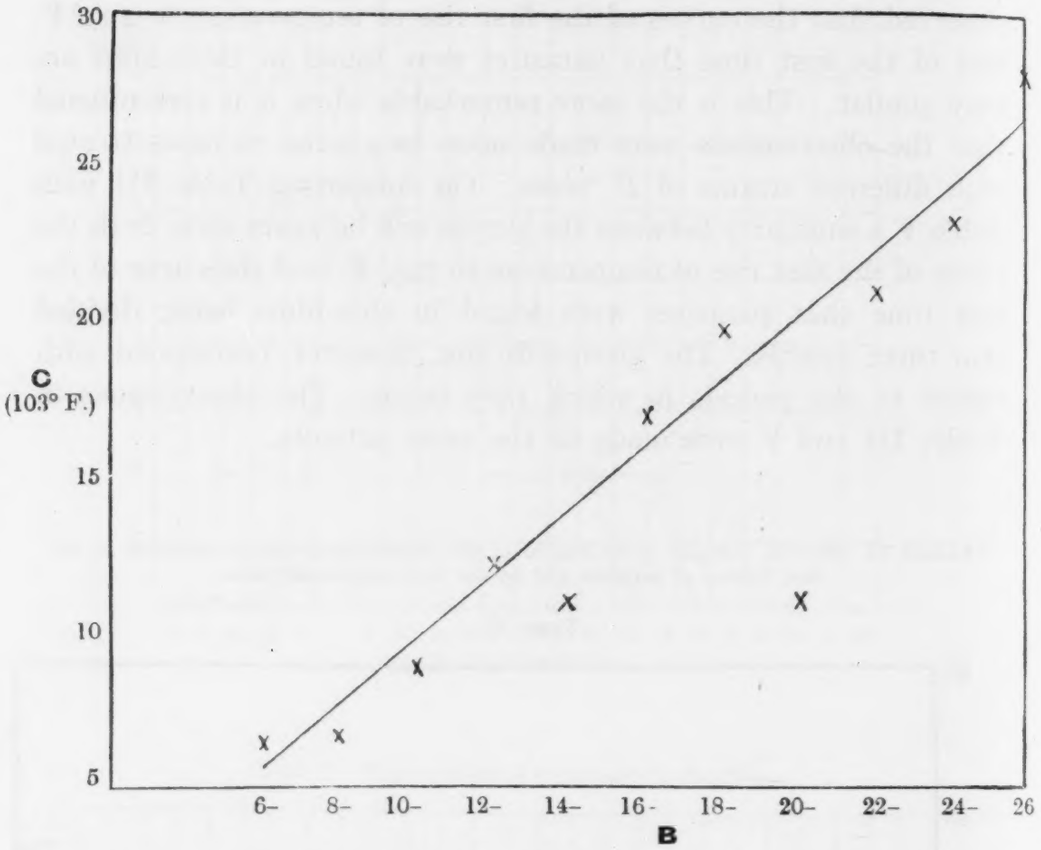


TABLE VII.



A. Number of days to the first rise of temperature to 101° F. expressed as averages of each group of *B*.

B. Number of days to the first finding of parasites. Cases arranged in two-day periods; e.g., patients in whom parasites were first found on the 6th and 7th days are grouped together in group 6–.

C. Number of days to the first rise of temperature to 103° F. expressed as averages of each group of *B*.

X = Average number of days before the first rise of temperature to 101° F. or 103° F., the diagonal lines are drawn to agree with the majority of the crosses.

If observations of the first time that parasites were found in thin-films, searched for varying periods, and of the first rises of temperature to 101° F. and to 103° F., in the same patients, are grouped, it will be seen that the means lie close to a straight line as shown in Tables VI and VII. These tables show that, in the same patients, there is a marked tendency for the parasites to be found for the first time when the first rise of temperature occurs. On working out the correlation-coefficients for the same variables a high correlation is observed. The coefficient for the first time that parasites were found and the first rise of temperature to 101° F. is + ·9032, and that for the same first variable but the rise of

temperature to 103° F. is + ·9091. There is, therefore, little difference between the correlation-coefficients when either the first rise to 101° F. or that to 103° F. is used.

The above confirms the observation that parasites are first found when the first rise of temperature occurs.

(iii) Intravenous inoculation. The duration of the incubation-period when this method is chosen would appear to be shorter than when the injection is made subcutaneously. Sir Ronald Ross (1911) gives details of six intravenous inoculations of benign tertian malaria. In these cases fever first appeared in from 3 to 12 days and parasites were first found in from 4 to 12 days after inoculation. Templeton (1924) states that in twenty cases of dementia praecox inoculated intravenously with 2 to 3 c.c. of malarial blood the temperature usually rose the day after inoculation. Macbride and Templeton (1924) found that pyrexia usually developed on the second or third day in a series of eighteen general paralytics. In both series of cases the temperature was, as a rule, irregular for a few days. Davidson (1925), in a series of sixteen general paralytics, found that the incubation-period varied from 4 to 19 days. The period was measured by the occurrence of fever and the first appearance of parasites.

Therefore, in 60 intravenous inoculations the incubation-period was found to vary from 1 to 19 days.

(iv) Intramuscular inoculation. Dr. D. R. Alexander has kindly supplied me with details of cases of general paralysis inoculated intramuscularly at Bexley Mental Hospital. The following table shows the length of the incubation-period as measured by the first rises of temperature. The strain of *P. vivax* utilised was the same as that used at Claybury Mental Hospital.

TABLE VIII

First rise of temp. occurring	101° F. to 102.9° F.		103° F. or over	
	No. of cases	Per cent.	No. of cases	Per cent.
Up to 10 days	9	60.0	8	53.3
From 11 to 20 days	6	40.0	6	40.0
From 21 to 30 days	0	0.0	1	6.7

On comparing the above table with Table I it will be observed that when the same strain of parasite is used there is a tendency for the incubation-period to be slightly shorter with intramuscular inoculation than with subcutaneous. On account of the relatively small number of cases, namely fifteen, in Table VIII it is probable that the differences between the lengths of the incubation-period with the two methods of inoculation are actually smaller than appears from the tables. This is in accordance with the findings of Davidson (1925). This observer noted that in a series of 13 cases the length of the incubation-period, as measured by the first fever and the first appearance of parasites, varied from 10 to 23 days, this approximating to the incubation-period when the subcutaneous route is adopted. In the two series of a total of 28 cases the incubation-period varied from 6 to 23 days as measured by the first occurrence of fever.

DOSAGE AND INCUBATION PERIOD

The usual dose of malaria-infected blood inoculated into general paralytics is from 1 to 5 c.c., although Pijper and Russell (1924) have used 10 c.c. There can, therefore, be great variations in the quantity injected. If one patient were inoculated with 2 c.c. of blood and a second with 4 c.c. it might be expected that the incubation-period of the former patient would be twice as long as that of the latter. But as the malarial parasite is the cause of the clinical signs of malaria it is clear that the volume of blood in itself can bear no relation to the incubation-period, but that the number of parasites present in the blood is the important factor. Therefore, in order to endeavour to determine whether there is any correlation between the number of parasites injected and the length of the incubation period it is necessary to know the actual, or the comparative, number of parasites in unit volume of blood. If the comparative number is chosen then the blood for inoculation must be drawn at one time from one patient, for the numbers of parasites vary in different cases, and also in the same case at different times.

The blood should then be divided and inoculated into the patients whose incubation-periods are to be compared. The blood must be well-shaken before each inoculation or the red cells containing the parasites will sink to the bottom and the patients will not receive the correct number of red cells according to the volume of blood injected. For the same reason no more than the exact quantity of blood required for each injection must be sucked into the syringe. If more than required is in the syringe, the first patient to be inoculated may receive too few or too many erythrocytes per cubic centimetre according to whether the needle of the syringe is held pointing upwards or downwards.

The above comparative method has been used in the study of a series of cases inoculated subcutaneously with benign tertian malaria at Claybury Mental Hospital. The following method was that adopted for determining the first appearance of the parasites. Thin-films were examined daily, commencing seven days after inoculation, except in certain cases in whom the rises of temperature started before that date. After the first appearance of parasites in the films had been found in this manner, more accurate observations were made. The films taken on the day previous to the first appearance of the parasites were each examined during a standard time of thirty minutes. Particular attention was paid to the edges and to the ' tags ' of blood at the end of the film as parasites are often found in greater numbers in these situations than in the remainder of the film. Table IX shows the results obtained. The patients bracketed together were inoculated from the same patient. The total quantity of blood required was withdrawn, divided into the necessary quantities, and injected into the general paralytics to be treated. The relationship between the quantity of blood, and therefore the comparative number of parasites inoculated, and the length of the incubation-period can therefore be studied in each series of cases bracketed together. The table shows the duration of the incubation-period as measured by the date on which the first rise of temperature to 101° F. and to 103° F. occurred.

TABLE IX

Series No.	Patients No.	Sex	Dose in c.cs.	In Days		
				Parasites first found	1st Temp. to 101° F.	1st Temp. to 103° F.
1	1	M	2	17	17	17
	1A	M	2	13	9	9
2	2	M	2	14	11	13
	2A	M	2	16	19	19
3	3	M	1.5	7	7	10
	3A	F	1.5	5	7	8
4	4	M	3	19	21	22
	4A	F	3	19	18	18
5	5	M	5	12	8	9
	5A	F	5	26	26	29
6	6	M	5	6	5	6
	6A	F	4.5	8	6	6
7	7	M	3	18	20	20
	7A	F	5	11	6	8
8	8	M	2.1	9	8	9
	8A	M	4	6	7	8
9	9	M	2	11	6	8
	9A	M	3	11	6	6
	9B	M	4	7	6	11
	9C	M	5	7	8	10
10	10	M	2	21	17	19
	10A	F	4	16	15	15
	10B	M	8	13	12	14

The above table may be divided into two groups: the first consists of the series Nos. 1 to 5, the patients in each series being given the same number of parasites; the second consists of the series Nos. 6 to 10, the patients in each series being given different

numbers of parasites. Series Nos. 1 to 4 show that when there is the same dosage of parasites the length of the incubation-period is somewhat similar in each series. In series Nos. 1, 2, and 4, it is more nearly similar when it is measured by the time that parasites were first found than by the first rises of temperature. In series No. 5 there is a marked difference between the length of the period in the two patients. Patient 5A had been inoculated previously with malaria but had not 'taken.' The resistance of this patient was presumably high. After the second inoculation, however, all the parasites could not have been destroyed but, if a large number were, the same effect would be produced as if a small number had been injected.

The later series of the table, Nos. 6 to 10, show the effect of inoculating different numbers of parasites. It will be observed that, in each series, the cases that received the smaller dose gave the longer incubation-period as measured by the first appearance of parasites. This was also found to hold when the length of the incubation-period is measured by the first rise of temperature except in one series, No. 9. In this series the patients that received the greatest number of parasites showed the longest incubation-periods as regards the first rise of temperature, but the shortest as regards the first appearance of parasites. It will also be observed that the incubation-periods measured by the first time that parasites were found agree more nearly with the dosage, in an inverse relationship, than do the same periods when measured by the first rise of temperature.

SUMMARY

(i) The incubation-period of the naturally-acquired benign tertian malaria is given by most authorities as being from 6 to 21 days with, however, wide variations.

(ii) In 34 cases (chiefly from the literature) inoculated by means of anopheline mosquitos, the incubation-period varied from 7 to 30 days as measured by the first date on which parasites were found, and, in 37 cases, from 7 to 25 days as measured by the first rise of temperature.

(iii) Subcutaneous inoculation of malarial blood gives, according to a number of writers, an incubation-period of from 1 to 32 days. A series of 50 general paralytics inoculated subcutaneously with *Plasmodium vivax* showed that 90 per cent. gave a rise of temperature of from 101° F. to 102·9° F. in less than 21 days after inoculation and 46 per cent. in less than 10 days. The first rise of temperature over 103° F. occurred within 21 days in 84 per cent. and within 10 days in 38 per cent.

(iv) Following subcutaneous inoculation there is a well-marked correlation between the first rises of temperature to 101° F. and to 103° F. and the first finding of parasites in thin-films. The frequency-curves of the first finding of parasites in thick-films and of the first rise of temperature to 101° F. are very similar, although the observations were made upon two different series of cases inoculated with two different strains of parasite. Curves of the first finding of parasites in thin-films and the first rise of temperature to 103° F., in the same series of cases, are also somewhat similar.

(v) Intravenous inoculation of malarial blood gave an incubation-period of from 1 to 19 days in a series of 60 cases collected from the literature.

(vi) In a series of 28 cases inoculated intramuscularly at Bexley and Winwick Mental Hospitals the incubation-period varied from 6 to 23 days.

(vii) In 10 series of cases injected subcutaneously with malarial blood it was found (*a*) that when similar numbers of parasites were injected the incubation-periods were of somewhat similar lengths, and (*b*) that when different numbers of parasites were injected the incubation-periods showed a marked tendency to be shortest when the dosage of parasites was the greatest. In one series of four cases this relationship did not hold as regards the first rises of temperature but only as regards the first dates on which parasites were found. In most series the length of the incubation-period as measured by the first time that parasites were found, under standard conditions, corresponded more nearly to the dosage than did the length of the period as measured by the first rises of temperature.

I have again to thank Lieut.-Col. S. P. James for his kind assistance. My thanks are due to Drs. G. F. Barham and G. Clarke, Medical Superintendents of Claybury and Bexley Mental Hospitals,

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THE MODE OF ACTION OF BAYER '205' ON TRYPANOSOMES

BY

I. J. KLIGLER AND I. WEITZMAN

(*From the Laboratory of the Malaria Research Unit, Department of Health, Haifa*)

(*Received for publication* 18 *May*, 1925)

Since its introduction the drug Bayer '205' has excited the interest of various workers engaged in the study of trypanosomes or of general chemotherapeutic problems. This interest is due partly to its active trypanocidal properties and partly to the peculiarity of its behaviour *in vivo* and *in vitro*. *In vivo* it excites a profound reaction, modifying the coagulability of the blood (Steppuhn, Zeiss u. Brychonenko, 1923) injuring the red blood cells (Sei, 1923), stimulating a lymphocytic response (Kligler & Weitzman, 1924), and, in larger doses, producing marked toxic effect on the kidneys (Duncan and Manson-Bahr, 1924). Unlike most other drugs it is retained in the body in active form for weeks after the injection (Mayer u. Zeiss, 1920, and Ruppert, 1923). Another striking peculiarity is the apparent difference in its trypanocidal power *in vivo* and *in vitro*; *in vivo* it is active in small doses, while *in vitro* it is apparently ineffective.

All those who have experimented with the drug agree as to its profound effect on the parasites in animals; but there is a considerable amount of controversy as to the mode of action on the trypanosomes. Morphological studies by Steffan (1922) and by Hesselbach (1922) indicated a direct effect on the cell protoplasm, and observations by Mayer and Zeiss (1920) and Shintake (1923) suggested an influence on the process of division. The work by Haendel and Yotten (1920) showed that there is a direct combination between the trypanosomes and the drug and that the latter cannot be released by washing. Ruppert (1923), on the other hand, concluded from his experiments that Bayer in its active form is not fixed *in vitro* although it does exert some effect, and that the action of the drug is indirect.

The nature of the action of the drug is of more than theoretical importance. If its effect is really an indirect one, it follows that the usual *in vitro* estimation of the parasiticidal property of a drug is of little value as an indication of the behaviour of the drug in the animal body. It seemed of interest, therefore, to investigate further the effect of the drug on the trypanosomes *in vitro* and to ascertain whether there is any relation between its effect *in vitro* and *in vivo.*

As our experiments were drawing to a close Nauck (1925) published a paper on the same subject. This article does not, therefore, present any new information, but our experiments serve to supplement as well as confirm Nauck's findings.

Nauck worked with a strain of nagana trypanosome and used mice as his culture medium; infecting rabbits, treating them with Bayer, and then, at varying intervals after treatment, infecting mice with the blood of the treated rabbit. Nauck used large doses of Bayer and carried on most of his experiments *in vivo.*

We used a strain of *Tr. evansi* and our procedure differed from Nauck's in that the exposure of the trypanosomes to the drug were made *in vitro* and only the effect on the organisms tested by inoculation into animals to determine loss of virulence. Our experiments were also designed to obtain an approximate quantitive comparison of the trypanocidal power of the drug *in vitro* and *in vivo.*

The following is a brief presentation of the principal experiments bearing on this question.

The object of the first series of experiments was to ascertain whether exposure to the drug in any way affected the virulence of the trypanosomes. After exposure of the organisms for varying lengths of times in varying dilutions of the drug, the trypanosomes were sedimented and injected into guinea-pigs or rabbits. Adequate controls were always made.

EXPERIMENT 1.—The first experiment consisted in exposing suspensions of trypanosomes in serum to which varying dilutions of Bayer were added. The suspension was kept three hours at 25° C. centrifugalized, the supernatant solution containing the drug was decanted, and the sediment inoculated into rabbits and guinea-pigs. The details of this experiment are given in the protocols.

Protocol a, Experiment 1. Date 23.9.24.

4 c.c. blood from a guinea-pig containing 4 trypanosomes per microscopic field, defibrinated; 1 c.c. saline added; centrifuged at 700 revolutions for 5 minutes; opalescent fluid withdrawn; divided into 3 parts; to 1 part added Bayer in concentration 1/200; to 1 part 1/400; 1 part-control; kept 3 hours at 25° C. (incubator); centrifuged; fluid decanted, sediment shaken in 1 c.c. saline, injected half into a rabbit (R.) and half into a guinea-pig (G.p.).

R. 12. Injected tryps. in Bayer 1/200; after 5 days positive.

R. 13. Tryps. in Bayer 1/400; after 9 days positive.

R. Control. After 9 days positive.

G.p. 38. Tryps. in Bayer 1/200, after 5 days positive.

G.p. 39. Tryps. in Bayer 1/400, after 10 days positive.

G.p. Control. After 9 days positive.

Protocol b, *Experiment* 1. Date, 2.10.24.

Guinea-pig punctured; numerous parasites; 3½ c.c. blood taken; defibrinated by means of beads; 1 c.c. saline added, centrifuged. To the plasma added Bayer to concent. 1/100, 1/200, 1/400; ½ c.c. quantum taken, kept 3 hours, centrifuged, serum decanted; to sediment added ½ c.c. saline and 0·2 c.c. injected into each animal.

R. 15. Bayer 1/100; negative, observed 37 days; 10.11.24 injected 10 c.c. oil; observed 18 days; negative; superinfected; positive after 7 days.

R. 16. 1/200; died after 5 days; intercurrent infection.

R. 16a. 1/400; died after 5 days; intercurrent infection.

R. 17. Control. Died after 5 days; intercurrent infection.

G.p. 41. Bayer 1/100; observed 37 days; results negative. 10.1.24 injected 4 c.c. oil, observed 18 days; negative. 18.11.24 superinfected; positive after 4 days.

G.p. 42. Bayer 1/200; negative; history same as g.p. 41.

G.p. 43. Bayer 1/400; positive after 14 days.

G.p. Control. Positive after 10 days.

5.10.24 preparation of material the same as that of 2.10.24 and injected again into

R. 18. 1/200.

R. 19. 1/400. To replace R. 16 and R. 16a.

R. 18. 1/200, negative; observed 35 days. 10.1.25, 10 c.c. oil; observed 18 days; negative; superinfected; positive after 5 days.

R. 19. 1/400 positive after 14 days.

It appears that contact of trypanosomes for three hours with a 1 : 100 dilution of the drug is sufficient to destroy their virulence; a 1 : 200 dilution gave variable results; in one experiment the organisms were still infective, in the other not; a three-hour exposure to 1 : 400 dilution did not completely destroy the virulence, but the incubation period was prolonged, indicating a certain degree of injury.

EXPERIMENT 2.—This experiment was similar to No. 1, except that the exposure was for twenty-four hours. The results as shown in the protocol were negative, even in a dilution of 1 : 400.

Protocol a, Experiment 2. Date, 13.10.24.

Two guinea-pigs punctured; 3½ c.c. and 2½ c.c. blood taken; positive 7 per field; defibrinated; 2 c.c. saline added; centrifuged; supernatant fluid containing tryps. withdrawn; Bayer added to dilution 1/200, 1/400 in quant. of ½ c.c.; fluid left for control. After 24 *hours* suspensions examined; tryps. alive; sluggish motion; tubes centrifuged; clear fluid decanted saline added to sediment and injected with glass capillaries intraperitoneally.

R. 25. 1/200; negative; observed 30 days. 15.11.24 injected 10 c.c. oil; negative. 28.11.24 superinfected; positive; 11 days incubation.

R. 24. 1/400; negative; observed 30 days; 15.11.24 injected 10 c.c. oil; negative. 28.11.24 superinfected; positive; incubation 9 days.

R. 25. Control; positive after 6 days.

Protocol b, Experiment 2. 12.1.24.

Two guinea-pigs bled; 3½ c.c. blood; defibrinated; centrifuged slow speed until fluid opalescent.

0.25 c.c. susp. of tryps. 0.25 c.c. 1/100 Bayer	0.25 c.c. susp. of tryps. 0.25 c.c. 1/200 Bayer	0.25 c.c. susp. of tryps. 0.25 c.c. saline
0.50 c.c. 1/200 After 24 hours at 25° C.—1/200; Alive; persistaltic movements of the undulating membrane. Motion sluggish.	0.50 c.c. 1/400 Control for motility; 1/400; Active movement.	Control Control; Active movement.

Tubes centrifuged 10 minutes at highest speed; clear serum decanted; added ½ c.c. saline to each tube; injected 0·2 c.c. into each animal.

R. 27. 1/200 negative; observed 44 days; superinfected; positive; 5 days incubation.

R. 28. 1/400 negative; after 44 days superinfected; positive after 5 days.

R. 26. Control; positive after 5 days.

EXPERIMENT 3.—This was a repetition of Experiment 1, namely, a three-hour exposure, but a smaller number of trypanosomes was injected; the results showed that even an exposure of three hours to 1 : 400 dilution of the drug renders the organisms non-infective.

G.p. 34. 3 c.c. (tryps. 1 per field) taken; defibrinated; added 1 c.c. saline; centrifuged; dilutions to 1/200, 1/400 Bayer made as in experiment date 12.1.25; kept 3·15 hours in incubator at 25° C.; centrifuged, clear fluid decanted; to sediment added ½ c.c. saline; shaken; 0·2 c.c. injected into each animal.

R. 34. 1/200 negative; observed 19 days; superinfected 1.3.25; positive after 7 days.

R. 35. 1/400; negative; observed 19 days; 1.3.25 superinfected; positive; incubation 5 days.

R. Control. Positive; incubation 5 days.

EXPERIMENT 4.—This experiment was a repetition of Experiment 2 (twenty-four hours exposure), except that higher dilutions of the drug were used (800 and 1,600). The results indicated that even as low a concentration of the drug as 1 : 1600 is sufficient to destroy the virulence of the organisms. The control trypanosomes in each case were put through the same manipulations as the drug-exposed organisms, so that the possibility of loss of virulence through mechanical injury was eliminated.

Protocol Experiment 4. 7.2.25.

Two guinea-pigs bled 2½ c.c.; tryps. 3 per field; defibrinated; added 1 c.c. saline; centrifuged at 750 revolutions; opalescent fluid still containing few r.b.c used. Dilutions with Bayer made; opalescent fluid taken; 0·9 c.c., ½ c.c., ½, etc., to the first tube added; 0·1 c.c. 10% Bayer; dilution obtained 1/100; ½ c.c. transferred to second tube; etc. Final dilutions 1/100, 1/200, 1/400, 1/800, 1/1600; tubes left for 24 hours in the incubator at 25° C. After 24 hours examined; 1/100—slight undulant movement; 1/200 sluggish movement
1/400 active movement
1/800 active movement
1/1600 active movement
Control active movement.

Tubes 1/800, 1/1600 and control; centrifuged; clear fluid decanted; sediment diluted in ½ c.c. saline; tryps. still active; injected 1/800 into rabbit 48; 1/1600 into rabbit 49; control into rabbit 50.

R. 48. Observed 30 days; negative.
R. 49. Observed 30 days; negative.
R. Control. Positive after 6 days; heavy infection.

This series of experiments showed that Bayer ' 205 ' has a marked effect on trypanosomes *in vitro*. Ordinarily this effect is overlooked because the result is judged by the motility of organisms. The motility is not, however, an index of protoplasmic injury and the principal effect of the drug lies in a lowering or destruction of virulence due presumably to cell injury.

On the basis of these experiments made *in vitro*, it appears that the action of the drug *in vivo* is also direct and that the therapeutic as well as prophylactic action of the drug depends on the concentration of the drug in the body and the rate of elimination by a given host.

Previous therapeutic experiments showed clearly that the drug is active in certain proportional doses, at least in so far as rabbits and guinea-pigs are concerned. A dose of 0·1 gm. per kilo cured all animals; 0·05 gms. per kilo gave about 80 per cent. cures, while 0·005 gms. per kilo was not effective.

This relation of dose to effect is further illustrated by the following experiment. The purpose of this experiment was to see whether an infection can be aborted by a dose of Bayer smaller than the therapeutic dose. As is seen from the protocol below, the abortive dose is the same as the therapeutic dose ; 0·05 gm. per kilo aborted the infection, while 0·005 gm. did not.

Protocol Experiment 5. 13.12.24.

Two rabbits of same weight infected 13.12.24 ; on 16.12.24 rabbit 015 given 0·005 gm. Bayer per kilo and rabbit 016 was given 0·05 gm. per kilo. R. 015, 28.12.24, blood positive. R. 016, blood negative ; observed 32 days and continued negative.

The next series of experiments dealt with the prophylactic property of the drug. The object was to ascertain whether there was any relation between dose and the duration of protection. In other words, we tried to determine the relation between concentration of the drug and prevention of infection.

Experiment 6. Three rabbits injected with different doses of Bayer and at varying intervals ; after treatment the animals were infected.

R. 017. 0·05 gm. Bayer injected 16.12.24 ; infected 35 days later ; negative.

R. 018. 0·05 gm. Bayer injected 16.12.24 ; infected 35 days later ; negative. Infected again after three months ; positive, after incubation of 15 days.

R. 019. Injected 0·1 gm. Bayer per kilo ; one month later infected ; negative ; reinfected after another month ; trypanosomes appeared in the circulation after a delay of three weeks.

This experiment indicates that Bayer apparently confers protection only so long as the drug remains in the body in a concentration sufficient to affect the parasites. This relation between concentration of drug and protection is further emphasised by the subsequent experiment.

EXPERIMENT 7.—The object of this experiment was to determine whether the minimal protective dose corresponds to the minimal therapeutic dose. In this experiment the infection was given within a week or two after the injection of the drug. It is evident from the results that a dose of ·005 gm. per kilo failed to give any protection just as this dose is devoid of any therapeutic effect.

R. 022. Given ·005 gms. Bayer ; two weeks later infected ; positive after ten days.

R. 023. Given 0·01 gm. Bayer per kilo and infection followed 8 days later ; results negative ; animal observed two months.

R. 024. Given ·005 gms. Bayer per kilo ; infected 10 days later ; positive after 7 days.

ANALYSIS OF EXPERIMENTS.—The various experiments described above bring out two facts. First that contrary to our previous belief that Bayer exerts little trypanocidal action *in vitro*, it appears that the drug has a marked effect on the cell so that a dilution of 1 : 1600 is sufficient to destroy the virulence of the organisms. The other fact is that, in rabbits at least, the therapeutic, abortive and prophylactic doses are similar.

It is difficult to make comparisons between *in vitro* and *in vivo* effect, because it is not possible to determine the amount of drug which remains in circulation. The work of Mayer and Zeiss indicates that the drug is bound in the blood stream, by the serum, and is thus retained for many weeks. If the weight of the blood is accepted as approximately 1/15 the total body weight, it is possible to make a rough estimate of the effective dilution of the drug in the circulation. Our experiments show that doses of 0·005 gm. per kilo, or a dilution of 1 : 3000, fails either to protect or cure an animal while 0·01 gms. per kilo, or a dilution of 1 : 1500, is effective in a proportion of cases. Even if we assume that only 50 per cent. of the drug is bound in the serum, the effective doses *in vivo* correspond fairly well with those *in vitro*. A still further correspondence is the fact that when small doses of the drug are given the trypanosomes disappear from the circulation only sixteen to eighteen hours after treatment.

The rational conclusion then is that the therapeutic property of Bayer ' 205 ' is due to a direct injury to the trypanosomes which renders them avirulent for the host and thus readily destroyed and eliminated. The difference observed in different hosts are probably due to the rate of elimination of the drug, or in other words, to the residual concentration of the drug in the circulation.

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ON A NEW CESTODE FROM NIGERIA

BY

T. SOUTHWELL.

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A single specimen of a cestode worm from the small intestine of a ' large grey eagle ' was obtained by Dr. Ll. Lloyd at Sherifun, Northern Nigeria, 24.12.24. The species is new and is described as follows :

LATERIPORUS FUHRMANNI, n.sp. (figs. 1-4)

EXTERNAL ANATOMY :—The worm was fragmented but apparently measured about 20 cms. in length ; its maximum breadth is 1 mm. It is composed of a very large number of segments, the posterior margins of which are imbricated ; the most posterior segments are gravid, somewhat bell-shaped and as long as broad. The genital pores are unilateral and are situated just in front of the middle of the lateral margin.

Head. The head is somewhat oval and measures about 450μ by 330μ. It is armed with a single crown of about fourteen hooks, each of which measures about 31μ in length.

FIG. 1. *Lateriporus fuhrmanni* n.sp. Head. × 75.

Neck. A neck is present but, owing to the fact that the worm was fragmented, its length could not be determined.

INTERNAL ANATOMY :—As only a single worm was available, details relating to the muscular, nervous and excretory systems were not investigated.

FIG. 2. *Lateriporus fuhrmanni* n.sp. Hooks. × 1125.

Testes. There are about twenty-five testes situated posteriorly, behind, and lateral to the ovary. In full development they have a diameter of about 50μ.

Vas deferens. The cirrus pouch is situated anterior to the vagina, and it varies a little in shape ; usually it is a cylindrical organ extending in the median direction to the excretory vessel ; its median extremity appears glandular. The vas deferens is a long coiled tube, situated in front of the ovary and surrounded with a mass of prostatic glands.

Ovary. This is a bilobed organ composed of large acini situated in front of the testes.

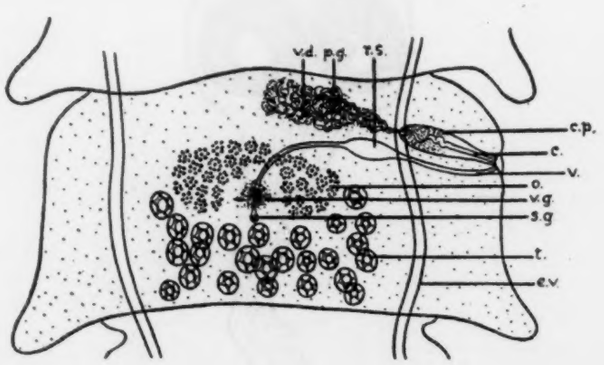

FIG. 3. *Lateriporus fuhrmanni* n.sp. Mature segment. *c.*—cirrus ; *c.p.*—cirrus pouch ; *t.*—testes ; *v.d.*—vas deferens ; *p.g.*—prostatic glands ; *r.s.*—receptaculum seminis ; *v.*—vagina ; *o.*—ovary ; *v.g.*—vitelline glands ; *s.g.*—shell gland ; *e.v.*—excretory vessels. × 75.

Vagina. The vagina runs posterior to the cirrus pouch and, immediately median to the excretory vessels, it dilates into a large pear-shaped receptaculum seminis.

The vitelline and shell glands lie immediately behind the ovary, the shell gland being very small.

Uterus. The uterus consists of a simple sac which completely fills the segment.

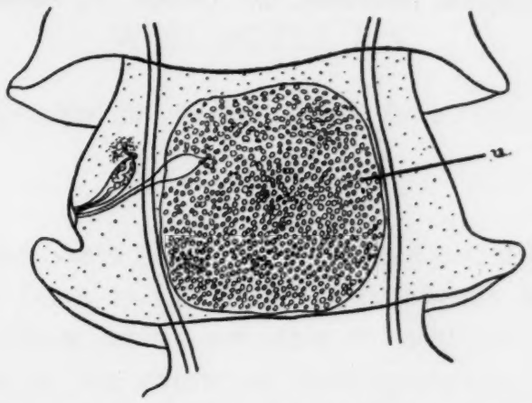

FIG. 4. *Lateriporus fuhrmanni* n.sp. Gravid segment. *u.*—uterus.

Eggs. No fully mature eggs were seen.

DIAGNOSIS. The single crown of hooks on the head, the unilateral pores, the posterior testes and the sac-like uterus place this worm in the genus *Lateriporus* Fuhrmann 1907. Six species of this genus are known.

The following table shows how *L. fuhrmanni* differs from the other species of the same genus, viz., principally in the size of the hook.

	Length of worm	No. of hooks	Size of hooks	No. of testes
cylindrica (Clerc, 1902) ...	25 mm.	16	200-216μ	15
teres (Krabbe, 1869)	42-60 mm.	12-16	150-170	30
biuterinus Fuhrmann, 1908 ...	300 mm.	16	120μ	16-18
spinosus Fuhrmann, 1908 ...	40 mm.	22	50μ	6 (?)
propeteres Fuhrmann, 1907 ...	several centimetres	16	120μ	about 12
geographicus Cooper, 1921 ...	172 mm.	?	?	15-20
fuhrmanni n.sp.	about 200	about 14	31μ	25

The type specimen is in the collection of the Liverpool School of Tropical Medicine.

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SOME CHARACTERISTICS OF THE FIRST STAGE LARVA OF *DERMATOBIA HOMINIS* GMELIN

BY

R. NEWSTEAD, F.R.S.,

AND

W. H. POTTS, B.A.

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PLATES IV AND V.

In Central and South America there is an Oestrid Fly, *Dermatobia hominis*, whose larva is the cause of cutaneous Myiasis in man and animals. These larvae are able to penetrate the unbroken skin, and there, in the course of development to maturity, give rise to tumours similar to those produced by the larvae of the Warble Flies (*Hypoderma* spp.) of Europe and North America. They are a source of considerable loss to cattle-owners, since the hides, riddled by the holes left on the emergence of the fully-grown larvae, are often valueless. According to Da Matta (1920), the proportion of hides thus damaged may be from 5 per cent. to as much as 70 per cent. The larvae are also indirectly responsible for the death of many animals, especially calves, since the tumours caused by them are liable to secondary infection from other myiasis-producing flies, whose larvae are unable themselves to pierce the unbroken skin. There is one fly of this latter type, the ' Screw-worm ' (*Chrysomyia macellaria*), which is very abundant in the Neotropical Regions and which in this way does a great deal of damage. Lastly, the *Dermatobia* larvae are the cause of much pain and inconvenience to man when passing through infected regions, and even give rise to serious illness if present in large numbers.

This Oestrid Fly differs from all other members of its class in that, instead of laying eggs or larvae directly on the hair or skin of the host, it lays batches of its eggs on the bodies of other insects, chiefly mosquitos. The larva of *Dermatobia* does not, apparently, leave the egg until the mosquito alights on a warm-blooded animal

to take a meal. Thus it would seem that the larva must be highly sensitive to a slight rise in temperature, and that the emergence from the egg must of necessity take place during the comparatively brief period in which the mosquito feeds. There is evidence to show that the larva, if unable to emerge completely from the egg during the time the mosquito is feeding, may withdraw itself into the egg and there wait until the mosquito visits another animal. This may occur several times, the larvae being capable of remaining alive for twenty days before reaching a host.

We are indebted to Dr. Nunez Tovar, who himself has done much to elucidate the remarkable life-history of the *Dermatobia*, for his gift of material which has enabled us to study these young larvae which penetrate the skin of their hosts. Before we present a detailed description of its characteristics, it has been thought well to give a short account of the life-history of this fly, as some interesting work has been done since the last comprehensive account in English was written by Sambon in 1915.

THE DISTRIBUTION OF THE FLY

According to Neiva and Gomes (1917), *Dermatobia hominis* occurs in Central and South America from Mexico to the Argentine. It appears to be absent from the United States, for, although cases infested with the larvae have been recorded from that country, it has always been found that the larvae were acquired in Central or South America. The fly seems to need a warm temperature, a certain degree of humidity, and forest country.

HOSTS OF THE FLY

The occurrence of larvae in the skin of man and various animals has long been known. The domestic animals in which they have been found are, in order of importance :—Cattle, dogs (especially hunting dogs), pigs, goats, turkeys, and, rarely, mules. There appears to be some doubt as to whether they occur in sheep, donkeys and horses. It is, in fact, sometimes stated that horses are not infested, but Neiva and Gomes (1917) give one record of the finding of larvae in a horse. The larvae have also been found in the

following wild animals :—Monkeys (whence the name ' Ver Macaque,' frequently given to the larva), jaguar, tapir, coati, agouti, deer (rarely), squirrels, and even birds, e.g., toucans and the ant-thrush (*Formicarius* sp.).

DISPOSAL OF THE EGGS

Although the existence of these larvae has been known for so long, there has been considerable doubt and uncertainty as to the exact way in which they reach the skin of their hosts.

Morales (1911), in Guatemala, was the first to publish a statement to the effect that the eggs were carried, firmly attached to the abdomen of a mosquito, *Psorophora* (then *Janthinosoma*) *lutzi*. From such eggs Morales obtained a larva, which produced in man characteristic tumours, and which presented all the characters of a *Dermatobia* larva. Tovar, in Venezuela, made similar observations two months earlier, but these were not published until 1913, in an article by Gonzales Rincones in the newspaper ' El Universel ' of Caracas.

These observations have since been confirmed by other observers, different flies being found to be *involuntary carriers* of the eggs in different parts of the country. Specimens of *Dermatobia* astride other flies have been caught, and even observed in the act of siezing the flies. According to Neiva and Gomes (1917), the adult *Dermatobia* frequent horses and other animals, and sieze flies which come either to suck blood or to feed upon sweat.

The final piece of evidence, the observation of the act of deposition of the ova by *Dermatobia*, has also been recorded. Neiva and Gomes (*loc. cit.*) enclosed adult females with various flies and found that eggs were laid on *Musca domestica*, *Stomoxys calcitrans*, and also on some Sylvan Muscoids. From these eggs larvae were obtained and were reared to the adult stage in dogs ; the whole process, from the laying of the eggs to the emergence of the adult, occupied 120 to 141 days.

Dr. N. Tovar also (1924) placed captured *Dermatobia* with specimens of the mosquitos—*Psorophora posticata*, *P. lutzi*, *P. tovari*, *Aedes trivittatus*, *Stegomyia calopus*, *Culex scapularis*, and Woodland Muscoids. Bundles of eggs were laid on all the examples of *Psorophora* (fifteen in all), irrespective of sex, whilst on none of

the others (twenty specimens in all) was a single egg to be found. In fact, he stated that although the insects other than *Psorophora* were sometimes seized by the *Dermatobia*, they were treated with violence and discarded damaged, whereas the *Psorophora* were always treated gently and liberated unharmed. He also records that *Dermatobia* eggs were never found in nature save on specimens of *Psorophora* (Plate IV, fig, 1).

In view of the results of modern investigations it is interesting to record some of the names by which the natives of various parts of America referred to the fly. For instance, in Venezuela the worm was commonly known as Gusano de Zancudo, in Colombia as Gusano de Mosquito, and in Trinidad as Ver Marangouin ; all of these terms mean ' Mosquito worm.' In an old book, the ' Historia del Nuevo Mundo,' written in 1653 by a Jesuit, Father Bernabe Cobo, the following statement, doubtless based on information received from the natives, is found :—' In some of the warm lowlands there is a species of mosquito . . . somewhat reddish. In each wound produced by this mosquito, soon grows within the flesh a spine-covered worm the size of a haricot bean or even larger . . . .' (Quoted from Sambon, 1915). Knab (1913) states that in 1905 the natives of the Isthmus of Tehuantepec, Mexico, pointed out to him certain large mosquitos (*Psorophora*) as ' Madre del Gusano.' In the first reference to this fly under the binomial system of nomenclature, that of Linnaeus Junior (1781), we find :—' . . . . the fly deposits on a man's skin, one after another, its eggs, or rather, its living larvae, *of which it carries about* 50 *on its hinder portion.*' (Quoted from Sambon, *loc. cit.*).

Da Matta (1920), in his account of *Dermatobia*, states that the mode of transference of the larvae may also be by direct oviposition on the skin of animals, or by indirect methods, by their deposition on leaves, from which the larvae may be picked up by passing animals, or by deposition on sweaty garments. These have been discussed by Neiva and Gomes (1917), For the first, we have been unable to find any record of a direct observation, either of eggs being found attached to the skin of animals, or of the act of deposition on the skin, although there is a record of *Dermatobia* having been seen hovering over horses with the ovipositor extended.

As to oviposition on leaves, there appear to be no records of

leaves having been found with ' packets ' of eggs adhering to them. Neiva and Gomes (*loc. cit.*) record that eggs were deposited on the sides of the vessel. They offer the explanation that, at a given moment, the female feels the necessity for oviposition irrepressible ; if then the insect which she is attempting to catch escapes, she oviposits on the nearest object. They found that eggs so laid, if kept in a moist place, produced larvae ; if, however, the conditions were dry, the eggs shrivelled and perished. They suggest that this may happen in nature, but the chances of larvae so produced being picked up by appropriate hosts do not seem to be very great. Since, however, it has been shown that the larvae can remain alive for twenty days in the egg without finding a host, this may be an alternative mode of transference.

Oviposition on ' sweaty ' clothes, too, seems to be supported by no direct observation. Neiva (1914) supports the hypothesis, saying that it would account for cases of infection of newly-born children who have never left the house. But he states that such cases are rare.

CARRIERS OF THE EGGS

The following insects have been found bearing batches of *Dermatobia* eggs in nature :—

BRAZIL :—*Psorophora posticata* (one example only, by Neiva and Gomes, 1917. Also by Peryassu, 1922).
Anthomyia heydenii (Lutz, 1917).
Anthomyia lindigii (Lutz, 1917).
Synthesiomyia brasiliana (Lutz, 1917).
Woodland Muscoids (on numerous occasions, Neiva and Gomes, 1917).

GUATEMALA :—*Culex* sp. unknown (Morales, 1911).

PANAMA :—*Goeldia longipes* (a non-bloodsucking mosquito, Shannon, 1925).

TRINIDAD :—*Psorophora* (then *Janthinosoma*) sp. (collected by Mr. F. Urich. Knab, 1913).

VENEZUELA :—*Psorophora lutzi*, *Psorophora posticata* (Tovar, 1924).

In captivity, *Dermatobia* has laid packets or batches of eggs on the following insects :—*Musca domestica*, *Stomoxys calcitrans*, Woodland Muscoids. (Neiva and Gomes, 1917). *Psorophora posticata*, *P. lutzi*, and *P. tovari* (Tovar, 1924).

Blanchard (1896) was sent the following flies by Da Silva Araujo in 1893, as being incriminated by the natives as ' parents of the Berne ' (*Dermatobia* larva) :— *Lucilia ruficornis* Macq., *Sarcophaga chrysostoma* Wd., *S. plinthopyga* Wd., and an *Hystricia.*

Neiva (1910) states that in Brazil nearly all species of *Tipulidae*, *Volucella obesa*, and a species of *Mesembrinella*, are accused of producing the warbles, whilst in Matto Grosso, several species of *Echinomyia*, and in Mexico, the beetle *Atractoceros brasiliensis* were also suspected. He, however, considered these popular beliefs to be erroneous.

Finally, Dunn (1918) has suggested the possibility of a tick (probably *Amblyomma cajannense*) being a carrier. The evidence is as follows :—Dr. Clark, in the course of two trips into the interior of Panama, discovered larvae of *Dermatobia* five times in wounds in man caused by tick bites. *Psorophora* were not obtained in collections of mosquitos from the places at that time, and besides, four out of the five sites were protected by clothing so that subsequent infestation of the wound seems improbable.

GENERAL DESCRIPTION OF FIRST INSTAR LARVA (Plate IV)

The general outline is somewhat elliptical, bluntly rounded anteriorly, and gradually attenuated posteriorly, the width of the last two segments being approximately half the width of the mid-thoracic segment, as seen in profile, after maceration in caustic potash (See fig. 1).

The cephalic segment is scantily clothed with very minute spines ; these appear to be more numerous dorsally and bilaterally (fig. 3A). The first thoracic segment bears a continuous band of relatively small and closely set black spines. The second and third thoracic segments are completely clothed with similar spines (figs. 3B and C). First, second and third abdominal segments show a double transverse series of large spines dorsally, and a single series ventrally ; the interspaces are set with smaller spines which are much more numerous in the posterior series than in the anterior one. The fourth to sixth segments inclusive are spineless. The seventh segment is clothed with long and slender, translucent spines (fig. 3D). The terminal segment is almost covered with relatively large strongly hooked and translucent spines (fig. 3E).

The spines on the thoracic and first three abdominal segments are directed backwards, whilst those of the last two segments are directed forwards. This arrangement of the posterior groups of spines enables the larva to retain a firm hold of the inner walls of the egg-shell after partial emergence.

The main tracheal tubes of the respiratory system, which resist the action of caustic potash, show very clearly (fig. 2). On the other hand the posterior stigmata are minute and not very clearly defined; they communicate with the tracheal trunks by two long and slightly narrower felt chambers which extend to the middle of the penultimate segment.

The antennal organs (fig. 2), presumably corresponding to the antenno-maxillary organs of other Dipterous larvae as described by Keilin (1915), are placed well forward in the cephalic segment in a dorso-lateral position; the proximal portion of the organ is strengthened with an incomplete band of dark chitin, the terminal portion being translucent.

THE MOUTH PARTS (Plate V)

The mouth parts consist of the following paired appendages:—

(1) Mouth hooks (*mh* in all figures).
(2) 'Prestomal sclerites' (*ps* in all figures).
(3) Stomal plates (*sp* in all figures).
(4) Membranous bands (*mb* in all figures).
(5) Rudimentary Hypo-pharyngeal sclerite (fig. 1C, *hs*).
(6) Cephalo-pharyngeal sclerites (*cs* in all figures).

(1) *The Mouth Hooks.*

These are highly chitinised, blackish and strongly falciform structures, the inner edge being finely though somewhat irregularly serrated. Proximally the anterior portion is strongly produced (figs. 1A, *mh*, and 1D). There are two centrally placed foramina.

(2) *The 'Prestomal sclerites.'*

These appear to consist of very thinly chitinised, translucent plates, which may act as a sheath to the tips of the mouth hooks. They are not apparent in fig. 1A, being hidden by the stomal plates, but they are indicated in fig. 1C, *ps*.

(3) *The Stomal Plates.*

These are relatively large cone-like processes, converging distally, and with longitudinal but somewhat indefinite ridges; proximally these structures are partly surrounded by a strongly chitinised plate, which is toothed on its distal or anterior margin (figs. 1A and B, *sp*.1, and fig. 1E). Below the cones is a mass of tissue with an irregular outline, portions of which seem to bear chitinous bodies, possibly muscle attachments.

(4) *Membranous Bands.*

These very thin and very slightly chitinised structures appear to arise towards the base of the mouth hooks; they are curved, and directed outwards and slightly backwards, the tips in some cases being slightly curved inwards, and somewhat strongly chitinsed.

(5) *Hypo-pharyngeal Sclerite.*

This consists of a median and very thinly chitinised plate with a pair of sub-median foramina, and lies between the anterior processes of the cephalo-pharyngeal sclerite, at the articulation with the mouth-hooks.

(6) *Cephalo-pharyngeal Sclerites.*

These consist of two plates, which are free dorsally, each consisting of three processes: a long, fairly heavily chitinised, anterior, inferior one, a short, fairly heavily chitinised, dorsal one, and a ventral one so lightly chitinised that it is difficult to see how far it extends into the thoracic region.

SOME AFFINITIES AND RELATIONSHIPS WITH OTHER FORMS

The most marked characteristics of the buccal organs of the first instar of *Dermatobia hominis*, are the presence of—

(1) the paired and well-developed mouth hooks (*mh*);
(2) the cone-shaped stomal plates (*sp* and *sp*1)

Another noteworthy feature is the absence of an unpaired median tooth, such as is found in most other first stage larvae, and is shown in *Hypoderma bovis* (Plate V, fig. 3, *mt*).

In his extensive paper on the larvae of Cyclorhaphous Diptera, Keilin (1915) states that in certain Acalyptrates, especially those with carnivorous larvae, one finds a precocious development of the paired mouth hooks of later stages. This condition also obtains in the first stage larva of *Calliphora*, where, however, as generally, a median tooth is present as well; *Hypoderma bovis* (Pl. V, fig. 3, *mt*, *mh*) shows both paired hooks and median tooth. No trace of the latter structure is to be seen either in *Dermatobia hominis* or in *Cordylobia anthropophaga* (Plate V, 2A-C), and this is paralleled in a figure given by Keilin of *Onesia sepulchralis* (*loc. cit.*, Pl. X, fig. 49C).

The paired mouth hooks of *Cordylobia anthropophaga* (' median buccal spine ' of Blacklock, 1923, fig. 1, 3C and D) show a very remarkable modification. These structures, of which four aspects are shown in our illustration (Plate V, figs. 2A-D) are broadly dilated unilaterally at the tips and strongly toothed on the distal margin (figs. 2A-C, *mh*, and fig. 2D). Seen in profile (fig. 2B) they are strongly directed upwards, and according to Blacklock lie, when at rest, at right angles to the cephalo-pharyngeal sclerite. These processes are also very strongly developed dorsally (figs. 2B and C, *mh* 3) and bilaterally are broadly expanded (fig. 2A, *mh*1). Further, ventrally there is a thin and broadly dilated flange (figs. 2A and C, *mh* 2) which appears to be connected with the finely spinose lower lip of the buccal cavity (fig. 2C, *bs*).

In the larva of *Hypoderma bovis*, which shows both median unpaired spines (fig. 3, *mt*) and paired mouth hooks (fig. 3, *mh*), the latter, as Carpenter and Hewitt (1914) have pointed out, are remarkable for being widely separated, directed laterally, and pointing outwards instead of upwards as in *Cordylobia anthropophaga*, or downwards and normally, as in *Dermatobia hominis*.

The peculiar form and high development of the structures we have called the stomal plates seem to be without exact parallel in other first stage larvae. They may be represented in a rudimentary form by some of the accessory pieces which Keilin has described (*loc. cit.*). For instance, there is an appendage which Keilin terms ' pièce en brosse ' (*loc. cit.*, Plate VIII, fig. 37, *f*, and *f* in other figures) which may be homologous, but as we have been unable to study these forms we cannot give a definite opinion as to their homologies.

Again there are the paired structures to which we have given

the term ' membranous lobes.' These are very indefinite structures. They appear in the case of *Hypoderma bovis* (fig. 3, *mb*) to correspond in position with the parastomal sclerites described by Lowne (1890) in the third stage larva of *Calliphora erythrocephala*, whilst in *Dermatobia hominis* they appear to be in a more anterior position, lying well in front of the bases of the mouth hooks.

It is interesting to note that in these three closely allied forms, all adapted to the same mode of life, quite different dispositions of the mouth parts exist. Thus the larva of *Dermatobia* penetrates the unbroken skin of man and animals, that of *Cordylobia* the skin of rats and sometimes man, and that of *Hypoderma* the tough hide of cattle. Of the three, *Dermatobia* perhaps approximates most closely to the condition shown by *Calliphora*, differing from it markedly by the absence of the median tooth, and by the strong development of the stomal plates; *Hypoderma* agrees with *Calliphora* in the possession of lateral hooks and median tooth, but differs in the disposition of these organs, the lateral hooks being directed outwards instead of downwards. *Cordylobia* is the most aberrant of the three, the mouth hooks being modified in a most extraordinary way, and directed dorsally instead of ventrally; the latter character is no doubt correlated with the mode of penetration of the larva in a horizonal direction under the skin, as has been well described by Blacklock (*loc. cit.*).

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EXPLANATION OF PLATE IV.

Dermatobia hominis.

FIG. 1. Adult mosquito (*Psorophora posticata*) carrying a batch of eggs of *Dermatobia hominis* on the ventral surface of the abdomen. Note that in one instance the embryo larva is shown partly protruding from the egg. × 10.

FIG. 2. Larva, First Instar. Lateral view. From a specimen macerated in caustic potash. Showing the arrangement of the dermal spines and the main sub-lying tracheal tubes. × 140.

FIG. 3A. Dermal spines from the cephalic segment.

FIG. 3B. Dermal spines from a thoracic segment.

FIG. 3C. Dermal spines from a thoracic segment.

FIG. 3D. Dermal spines from the penultimate abdominal segment.

FIG. 3E. Dermal spines from the terminal abdominal segment.

Figs. 3A-3E (inclusive) × 500.


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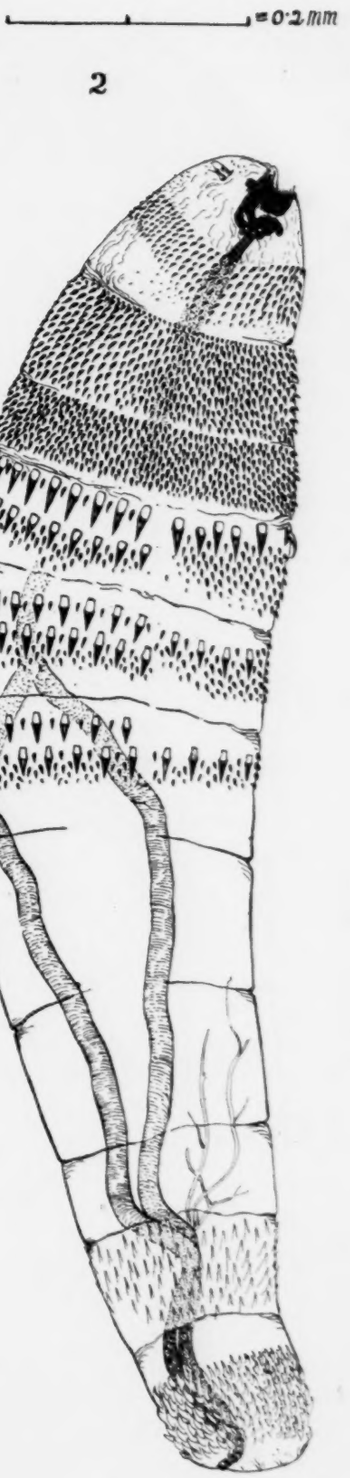
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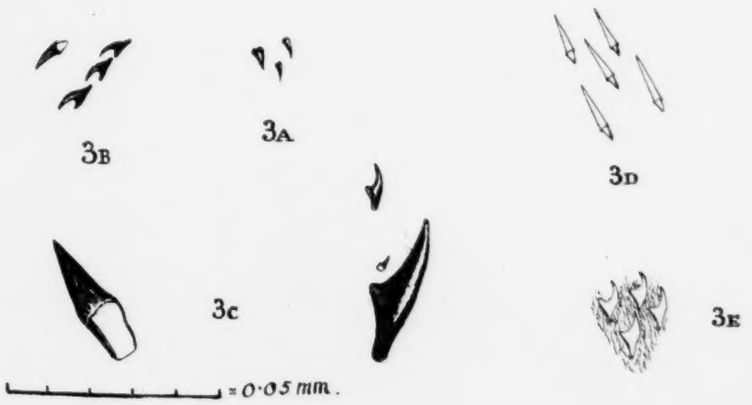
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EXPLANATION OF PLATE V.

Mouth Parts of First Instar Larva of Dermatobia hominis.

FIG. 1A. Profile.

FIG. 1B. Ventral.

FIG. 1C. Dorsal.

FIG. 1D. Mouth hook.

FIG. 1E. Two views of the proximal portions of the stomal plates, detached from the cone-shaped process.

Mouth Parts of First Instar Larva of Cordylobia anthropophaga.

FIG. 2A. Ventral.

FIG. 2B. Dorsal.

FIG. 2C. Profile.

FIG. 2D. Terminal portion of mouth hooks, ventral view, showing the arrangement of the teeth × 1000.

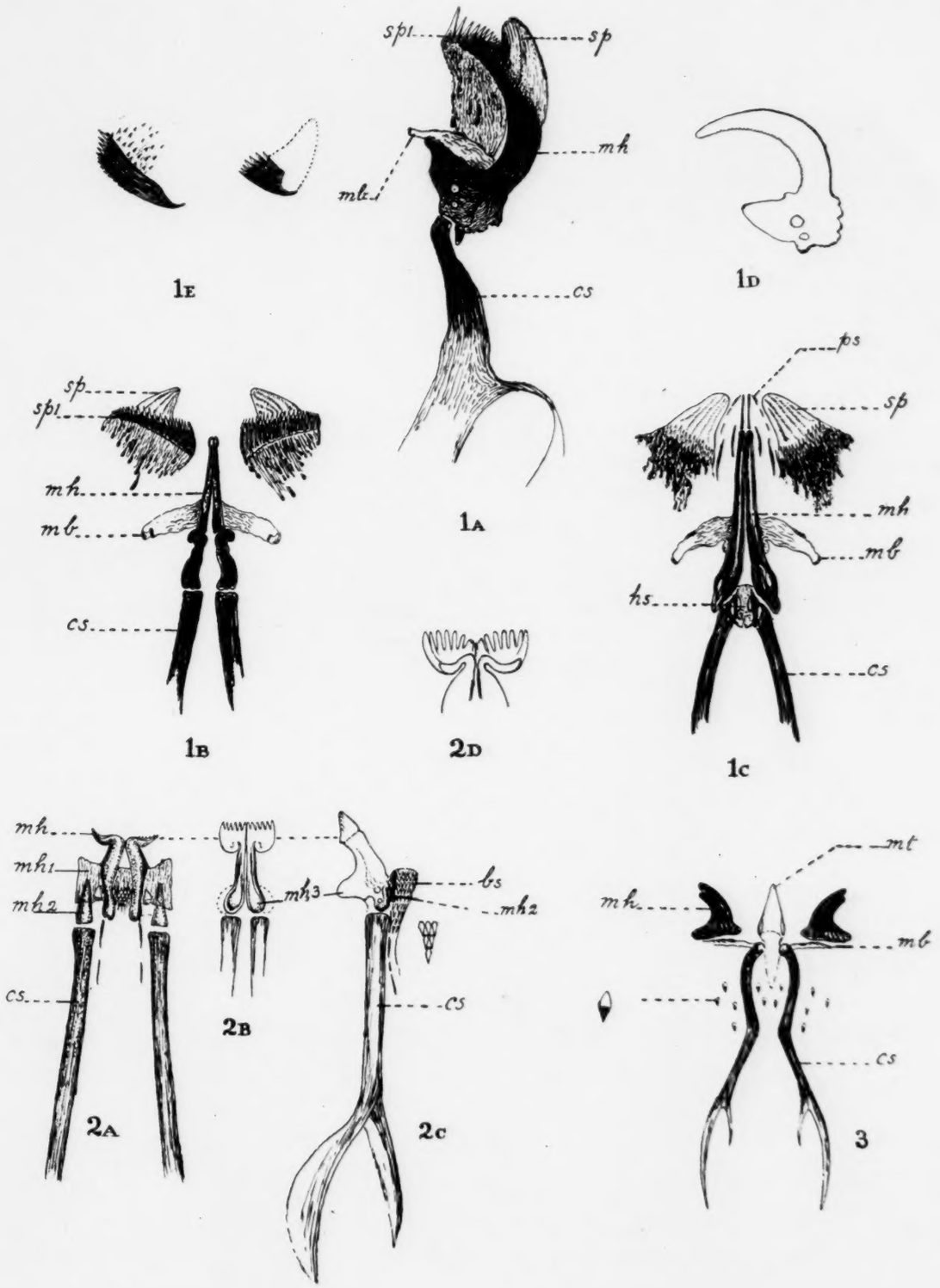
Mouth Parts of Third Instar of Hypoderma bovis.

FIG. 3. Dorsal.

All the figures with the exception of Fig. 2D. × 500.

EXPLANATION OF LETTERING.

cs = cephalo-pharyngeal sclerites.
bs = buccal spines.
hs = rudimentary hypo-pharyngeal sclerite.
mb = paired membranous bands.
mb = mouth hooks.
*mb*1 = lateral proximal extension of the mouth hooks.
*mb*2 = ventral proximal flange of the mouth hooks.
*mb*3 = dorsal proximal process of the mouth hooks.
mt = median tooth.
ps = prestomal sclerites.
sp = stomal plates.
spl = proximal pieces of stomal plates.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MISCELLANEA

PLACOBDELLA PARASITICA

Dr. Aitken Clark has presented to the Liverpool School of Tropical Medicine a large leech measuring, when fully expanded, about seven inches, which was found sucking human blood, in Para, Brazil.

The specimen proved to be *Placobdella parasitica* (Say) 1824. It is most commonly found attached to species of *Chelydra*, etc.

T. SOUTHWELL.

A CASE OF EMPYEMA SIMULATING ABSCESS OF THE LIVER

The patient, a male aged thirty, Brazilian, who had resided in Amazonas for the past ten years, was admitted to hospital 29.12.21.

History.—Patient dates his present illness from an attack of dysentery six months ago. Symptoms began with pain in the right hypochondriac region, debility, fever every afternoon, profuse sweating, loss of weight and swelling of the abdomen.

On admission.—Patient very much emaciated; abdomen generally distended, but markedly so in the right hypochondriac and epigastric regions. The right hypochondrium is occupied by a swelling, resistant, somewhat resilient, dull on percussion, reaching almost to the level of the right anterior spine of the ilium and across the mid-line; the swelling bulges in the right flank. Dullness on percussion is complete as high as the right nipple in front, and almost to the inferior angle of the scapula posteriorly. No fluctuation is perceptible over the swelling, which moves slightly with respiration.

No cough; no sputum; dullness and faint breath sounds over the base of the right lung as high as the inferior angle of the scapula.

Treatment and Progress.—On 31.12.21 an exploratory puncture was made one inch below the right costal margin in the anterior axillary lines, in the most prominent and most tender part of the tumour. One-and-a-half litres of reddish brown pus were aspirated with great relief of symptoms. Although no amoebae were present, on the assumption that the condition was probably liver abscess, a

grain of Emetine was administered hypodermically, and repeated daily till 21.1.22. The evening temperature was normal for a week, but then rose again. Another litre of pus was aspirated, but again two days later the temperature rose. It was then considered advisable to operate. On 21.1.22 a needle was inserted one inch below the costal margin in the anterior axillary line and a small amount of pus aspirated with a syringe. Under chloroform anaesthesia an incision was made, four inches long, parallel to the costal margin, its centre being just below the insertion of the needle. When the incision was deepened it was seen that the needle penetrated the diaphragm, which bulged down below the level of the incision. The diaphragm was incised and two litres of pus were evacuated. A finger passed through the incision and upwards encountered a large empty space, with the lung collapsed towards the apex. Inferiorly the diaphragm, partly adherent to the upper surface of the liver, was found to bulge downwards, below the level of the skin incision, to a distance of about two inches. A drainage tube was inserted and the remainder of the incision closed. For a week a fair amount of pus was discharged, the patient being encouraged to do breathing exercises to expand the lung.

2.2.22. Discharge much diminished ; drain and stitches removed.

5.3.22. Patient left hospital ; practically no discharge ; feeling very well and weighing 20 kilos more than on admission.

15.3.22. Patient returned to report. In the interval he had been riding on horseback every day. Wound closed and patient feeling very fit.

R. M. BURNIE.

THE GOLUBACSER FLY

'. . . There is, in Servia and the Banat, a minute fly,* from whose destructive assaults on the cattle the inhabitants have suffered immense losses. A traveller, arriving at Golubacs, on the Danube, thus speaks of it :—

" Near this place we found a range of caverns, famous for producing the poisonous fly, too well known in Servia and Hungary

* *Simulium columbaschense* Köll.

under the name of the Golubacser fly. These singular and venomous insects, somewhat resembling mosquitos, generally make their appearance during the first great heat of the summer, in such numbers as to appear like vast volumes of smoke. Their attacks are always directed against every description of quadruped, and so potent is the poison they communicate, that even an ox is unable to withstand its influence, for he always expires in less than two hours. This results, not so much from the virulence of the poison, as that every vulnerable part is simultaneously covered with these most destructive insects; when the wretched animals, frenzied with pain, rush wild through the fields till death puts a period to their sufferings, or they accelerate dissolution by plunging headlong into the rivers" '*

'The Romance of Natural History,' by P. H. Gosse, F.R.S., 2nd ed., 1861, p. 111. Compare these ANNALS, XVIII, No. 3, 1924, p. 323.

J. W. W. STEPHENS.

* Spence's *Travels in Circassia*, i, p. 59.

ON *PROTEOCEPHALUS MARENZELLERI*, *P. NAIAE* AND *P. VIPERIS*

BY

W. N. F. WOODLAND

Wellcome Bureau of Scientific Research, Endsleigh Gardens, London, N.W. 1

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PROTEOCEPHALUS MARENZELLERI (Barrois, 1898)

This species, one of the largest known Proteocephalids, was first proposed (as *Ichthyotaenia marenzelleri*) by Barrois (1898) who supplied a very brief account of its structure from material collected by Calmette in 1897 from *Ancistrodon piscivorous* Holbr., the 'Water Viper,' a snake found in the southern United States. Ten years later, Schwarz (1908) published a more complete description, with three figures, solely based, however, upon the identical material studied by Barrois. Five years later still, Beddard (1913b) supplied some further details of structure from the examination of a number of immature specimens (the longest measuring about 250 mm.) found in a water viper which had died in the London Zoological Gardens. Since Beddard's specimens were immature and La Rue (1914) expressly recommends a further study of new material, the following description of the anatomy of one large fully-mature example, which I have found recently in the Wellcome Bureau collection of Helminths and which was collected from a water viper which had also died in the London Zoological Gardens, is worth publishing.

My single specimen measured between 300 mm. and 400 mm. in length and was well preserved in spirit. It shows well a striking feature of this species, viz., the very small proportion of the strobila which consists of mature and ripe proglottides. As Beddard remarks concerning his specimens, 'in proglottides situated 8 inches or so

from the scolex [the largest worm being 10 inches in length in spirit] there were merely traces of the reproductive organs,' and in my own single specimen, though it must have measured about 350 mm. (i.e., over 14 inches, in spirit) in total length, yet I did not obtain from the strobila more than about a dozen ripe proglottides and as many which could be described as mature, the rest of the strobila consisting of immature proglottides.

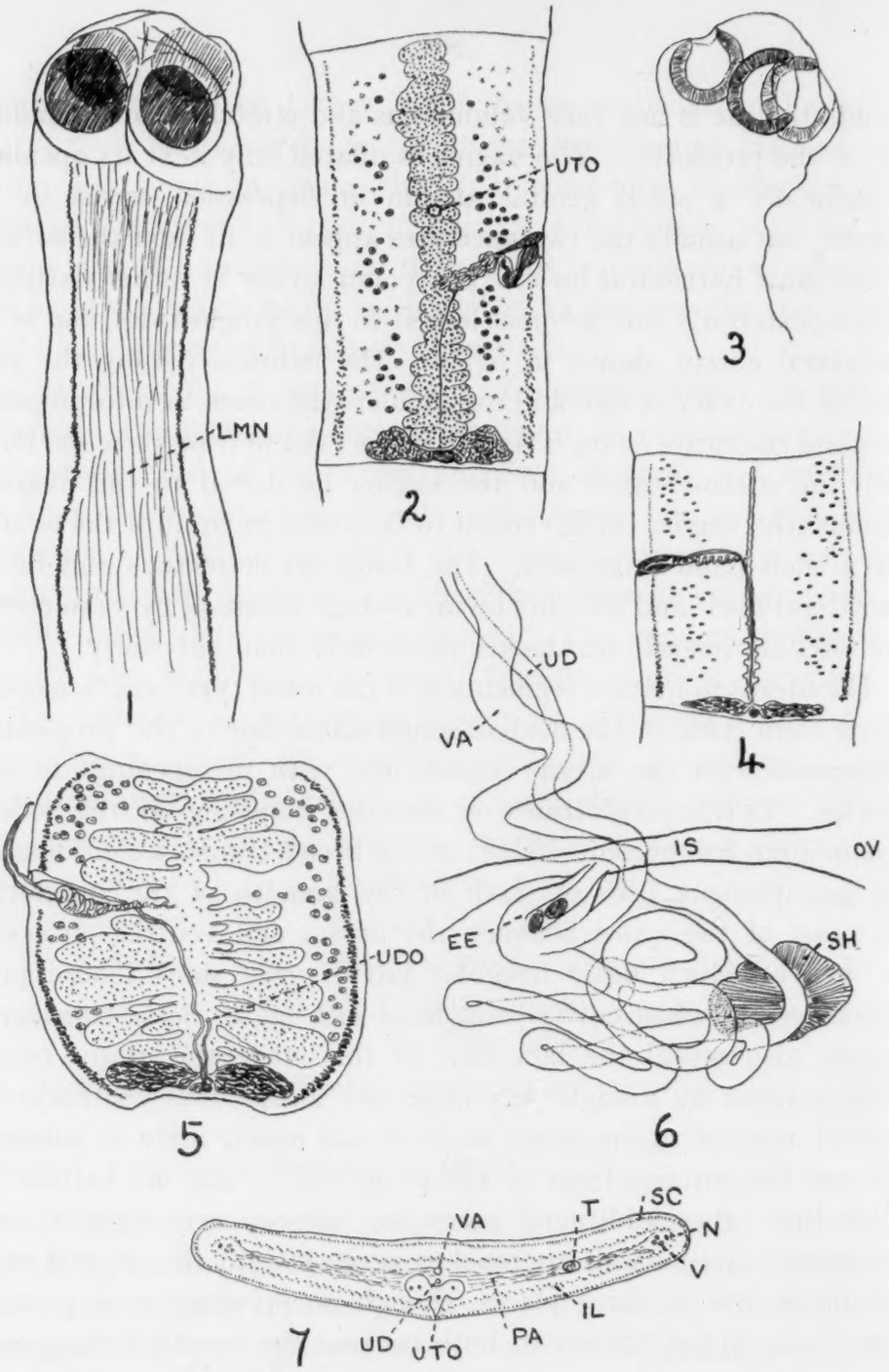
The scolex (fig. 1) was present and measured about 1·9 mm. in breadth. The four large suckers are borne on protrusible lobes on the anterior end of the scolex and face upwards and outwards, the apex of the scolex being quite insignificant, i.e., no ' rostellum ' is present. I did not sectionize the scolex to ascertain if a minute apical organ were present. The suckers measure about 0·913 mm. in breadth. Spinelets are entirely absent. The unsegmented neck is about 7 mm. long, with an average breadth of about 1·4 mm.

The strobila is, in transverse section, extremely flat and has a maximum breadth of a little over 3 mm. (3·068 mm.). The immature proglottides which, as already stated, compose by far the greater part of the strobila, vary in size and shape from $\frac{1\cdot9 \text{ mm. broad}}{0\cdot767 \text{ mm. long}}$ in front to $\frac{3\cdot068 \text{ mm. broad}}{1\cdot180 \text{ mm. long}}$ behind, and thus are all broader than long. Mature proglottides are more square in shape ($\frac{2\cdot95 \text{ mm. broad}}{1\cdot71 \text{ mm. long}}$ and $\frac{2\cdot8 \text{ mm. broad}}{2\cdot0 \text{ mm. long}}$), while ripe proglottides are, more anteriorly, distinctly broader than long ($\frac{3\cdot068 \text{ mm. broad}}{1\cdot8 \text{ mm. long}}$) and, more posteriorly, longer than broad ($\frac{2\cdot124 \text{ mm. broad}}{4\cdot0 \text{ mm. long}}$). The genital apertures are, as usual, irregularly alternate and open midway in the lengths of the proglottides, and the cirrus and vaginal apertures irregularly alternate as to which is anterior. The cirrus sac in mature and ripe segments is extremely broad antero-posteriorly, measuring 0·448 to 0·680 mm. in length and 0·149 to 0·298 mm. in maximum breadth. In some proglottides the sac stretches over a quarter of the breadth of the proglottis but usually a somewhat less distance. The contained cirrus is, next the opening, bulbous in form, and continuous with a straight portion which is connected with several coils of the ductus at the base of the sac. The cirrus was not everted in any of my preparations. The vas deferens

outside the sac is not very voluminous and stretches to the middle line of the proglottis. The vagina is dilated only next its opening. Occasionally a small genital atrium or depression seems to be present, but usually the two apertures appear to lie next the surface at the same horizontal level. The young ovary is rather flattened antero-posteriorly but becomes less so in ripe proglottides, and is of the lateral extent shown in fig. 2. The isthmus joining the two lobes of the ovary is thin and canalicular when seen in toto-preparations and the entire ovary is seen to be very flat in transverse sections. Both the uterine canal and the vagina lie dorsal to the ovarian isthmus (the vagina being ventral to the canal in front of the ovary) and a shell-gland is present. The testes are numerous and lie in two lateral fields and measure on an average about 44 by 22 microns. The vitelline strands are thicker posteriorly than anteriorly.

The uterus in mature segments is of the usual type, viz., a narrow hollow stem lying in the median longitudinal line of the proglottis, continuous with the uterine canal, and with no openings to the exterior. In ripe proglottides, on the other hand (fig. 2), the hollow median stem has become dilated into a broad trunk of considerable size (occupying a fifth or sixth of the breadth of the proglottis and most of the space between the dorsal and ventral surfaces), the wall of which bears irregular lateral very short lobose protuberances, the whole cavity being filled with eggs. Serial transverse sections also reveal the fact that at this stage the uterus opens to the exterior by a single very large and conspicuous ventral pore, situated anterior to the cirrus sac level and nearly midway between this and the anterior limit of the proglottis. I am not certain as to whether other additional pores are subsequently formed (and the ventral uterine wall approaches very close to the ventral subcuticula in two or three places, though no openings were present in my sections) but I doubt it, both because the proglottides appear to be fully ripe and because of the large size of the very well-defined single existing pore. The uterine eggs measure about 22 microns in external diameter, the embryos about 11 microns.

In transverse section the mature and ripe proglottides are seen to be extremely flat. Beddard remarks that in his immature specimens he could find ' no marked layer of longitudinal fibres in the body generally ' and quotes Schwarz as saying that ' die innere



FIGS. 1, 2. *Proteocephalus marenzelleri.*

„ 3 to 7. *Proteocephalus naiae.*

Fig. 1 (× 12). Scolex. Compare the magnifications of this and fig. 2, and figs. 3, 4 and 5.

Fig. 2 (× 12). Ripe proglottis. Dorsal view. The testes are degenerating. Note the small size of the uterine diverticula.

Fig. 3 (× 87·5). Scolex in outline.

Fig. 4 (× 12). Mature proglottis, unflattened. Dorsal view.

Fig. 5 (× 12). Ripe proglottis, much flattened. Dorsal view.

Fig. 6 (× 180). Ducts in the region of the ovarian isthmus. Ventral view.

Fig. 7 (× 27·5). Transverse section through a young ripe proglottis immediately in front of the ovary.

EE.—egg-ejector (' schluckapparat ') ; *IL.*—internal layer (sheath) of longitudinal muscles ; *IS.*—isthmus of ovary ; *LMN.*—longitudinal muscles of neck ; *N.*—nerve ; *OV.*—ovary ; *PA.*—modified parenchymal core in medulla ; *SC.*—nuclear layer of subcuticula ; *SH.*—shell gland ; *T.*—testes ; *UD.*—uterine canal ; *UDO.*—opening of uterine canal into median chamber of uterus sac ; *UTO.*—opening of uterus to exterior ; *V.*—vitellaria ; *Va.*—vagina.

Längsmuskulatur ist schwach.' In all my sections through mature and ripe proglottides there is a very distinct layer of internal longitudinal muscles—much more distinct than that in Beddard's '*Solenotaenia*' *viperis* (*vide infra*), because definite bundles of two, three or more fibres are present and are relatively numerous. The medulla contains a core of specialized parenchyma in which the meshes are transversely elongated. I only observed the specialized longitudinal musculature of the neck region in a toto-preparation.

PROTEOCEPHALUS NAIAE (Beddard, 1913)

Syn., *Ophidotaenia naiae* Beddard, 1913.

Of this species I possess more than two dozen specimens, all taken from the anterior and middle intestines of ten (fourteen examined) full-sized cobras (*Naia tripudians*), supplied by snake-charmers of the United Provinces, India. This species has already been described by Beddard (1913a) from three specimens obtained from a cobra which died in the Zoological Gardens, London, but a re-description is necessary owing to the original account being deficient in some respects. Most of my specimens were found in the intestine just behind the stomach and isolated detached proglottides were also found in the faeces on several occasions. My largest (unflattened) specimen measured 180 mm. in total length, with a maximum breadth of 2·5 mm., but other specimens (mostly without ripe proglottides) measured considerably less and one immature specimen only measured 43 mm. Beddard's largest specimen measured 110 mm., with a maximum breadth of 1·5 mm.

In the following account of the species I shall for the most part only deal with those features which require a more complete description than Beddard has given, or which, in my opinion, have been misunderstood by him. As Beddard remarks, the 'rostellum' (by which term I mean simply the terminal part of the scolex, anterior to the suckers) is never very conspicuous (fig. 3) and when retracted consists solely of a very restricted non-projecting area lying between the suckers. In my specimens (toto- and in sections), as in Beddard's, an apical body is absent, though a number of gland-like cells appear to be clustered in the position normally occupied by

the apical organ. Cuticular spinelets were absent. In seven of my balsam preparations, the scolex measured 0·248 to 0·303 mm. in breadth and 0·153 to 0·201 mm. in length (from apex to lower edge of suckers), and the maximum diameter of the suckers varied between 0·106 mm. and 0·153 mm. The suckers are borne on lobes of the scolex base (each sucker, however, occupying the bulk of the lobe) and are undoubtedly protrusible. The unsegmented neck in my specimens is of considerable length, varying between 3·5 mm. (in one case) and 6 mm. (in most cases) according to the state of contraction, and 0·116 to 0·614 mm. in breadth. According to Beddard the neck is ' short.'

The mature and ripe proglottides are of considerable size and are only found in the extreme hind regions of most worms, the greater part of the strobila being composed of large and yet immature proglottides. Only in one of my two dozen specimens were the proglottides in a ripe condition. The immature proglottides (unflattened) in most worms varied between $\frac{2{\cdot}242 \text{ mm. broad}}{0{\cdot}295 \text{ mm. long}}$ and $\frac{1{\cdot}770 \text{ mm. broad}}{1{\cdot}121 \text{ mm. long}}$, and more or less mature proglottides between $\frac{2{\cdot}655 \text{ mm. broad}}{0{\cdot}413 \text{ mm. long}}$ (considerable contraction) and $\frac{0{\cdot}531 \text{ mm. broad}}{3{\cdot}009 \text{ mm. long}}$ (considerable extension), but the average shape of the mature and ripe proglottides is square or a little longer than broad (figs. 4, 5). The genital openings are irregularly alternate and open, often on a distinct projection, either midway in the length of the proglottis or a little anterior to this point. The cirrus sac and vagina irregularly alternate as to which is anterior. The cirrus sac, in unflattened and not unduly contracted or extended proglottides, extends across about a quarter of the breadth of the proglottis and, when fully developed, measures about 0·498 to 0·531 mm. long and 0·083 to 0·107 mm. broad. In flattened (between glass slides) and very contracted or extended proglottides the sac varies enormously in size, from being almost globular in form and therefore very short, to very elongated in form and extending across at least one-third the width of the proglottis. In the cirrus sac both the cirrus (unarmed) and the ductus are usually coiled. In many of my flattened proglottides the cirrus is everted to its full extent and in some cases is longer than one-third the width of the proglottis,

and then the sac is practically invisible, from which I conclude (though the eversion may have been due to the artificial flattening) that, in these cases at least, the cirrus sac itself has been everted, though Beddard says that he has seen no evidence of this. The wall of the cirrus sac is thin but muscular. A small cloaca genitalis is present. The coils of the vas deferens in unflattened preparations are not very voluminous and are just visible as far as the middle line of the proglottis. In elongated proglottides the vas deferens coils form a bunch in the middle line. The vagina opens at the same horizontal level as the cirrus, but away from the opening it lies ventral to the cirrus sac and to the vas deferens coils. The vagina is very slightly dilated near its opening but in no other region and, except in very elongated proglottides, is sinuous or slightly convoluted just anterior to the ovary. The testes are about 120 in number and in unflattened preparations measure, on the average, about 62 by 36 microns. They are situated in two quite separate lateral fields. The vitellaria (cir. 14 by 11 microns) are, as usual, arranged in two thin lateral strands, which, however, are distinctly broader posteriorly than anteriorly. The ovary consists, as usual, of two lobes connected medianly by an isthmus. In surface view the lobes are narrow antero-posteriorly and extend laterally, in mature proglottides, only a little more than half-way to the proglottis edge. In sections they are seen to be very thin dorso-ventrally and to lie nearer the dorsal than the ventral surface of the strobila, though the isthmus connecting the lobes bends ventrally to allow of the dorsal passage of the uterine canal and vagina. The lobes are distinctly follicular. Beddard says that he ' could not find any signs of a shell-gland ' and he endeavours to correlate its supposed absence with the presence of a ' glandular investment ' on the walls of the uterine diverticula, which he assumes to take the place of a shell-gland. For my part, I have had no difficulty in finding a very distinct shell-gland (diagrammatically represented in fig. 6) in most of my preparations, and, on the other hand, I have been unable to find any investment of the uterine walls with cells which can be described as glandular. A distinct egg-ejector (' schluckapparat ') is also present. The uterus in my ripe proglottides (fig. 5) consists of (*a*) a wide median uterine sac extending the whole length of the proglottis from the ovary anteriorly, which carries on either side from 16 to 25 lobose diverticula

of very different sizes (I could not detect any diverticula ventral to the ovary), and (*b*) a uterine canal, which, with the vagina, passes *dorsal* to the ovarian isthmus, and opens into the median uterine sac some distance in front of the ovary. Nearly all the eggs are collected in the diverticula, in which they are freely scattered (not in clusters), and they possess two distinct shells, the outermost thick shell measuring about 25·6 microns in diameter, and the contained embryo 9 to 11 microns. The median sac of the uterus has a number of pointed downgrowths (fig. 7) which open on what is usually considered to be the ventral surface of the strobila. The position of the uterine pores is indeed the sole certain criterion of determining the orientation of the strobila.

In transverse sections (fig. 7) through mature proglottides the usual two layers of longitudinal muscles are to be seen—a very thin layer just external to the nucleated region of the subcuticula and internal to an equally thin circular muscle layer, and a thicker, though somewhat attenuated, internal layer of longitudinal muscles, demarcating the cortex from the medulla. The parenchyma is in most regions of a uniform wide-meshed character, but in the centre of the medulla there is a kind of core of closer-meshed parenchyma, with the meshes transversely elongated. This core of differentiated parenchyma is apparently identical with that figured by Beddard (1913b, p. 167, text-fig. 38) for '*Ichthyotaenia* sp.' (i.e., *P. marenzelleri*) only Beddard assumes (in the absence in his immature specimens of an internal longitudinal muscle sheath) that it represents the whole of the medulla, whereas in *P. naiae* and in my mature specimens of *P. marenzelleri* (*vide supra*) it is obviously only the internal region of it. I am ignorant of the significance of this altered parenchymal core.

PROTEOCEPHALUS VIPERIS (Beddard, 1913)

Syn., *Solenotaenia viperis* Beddard, 1913.

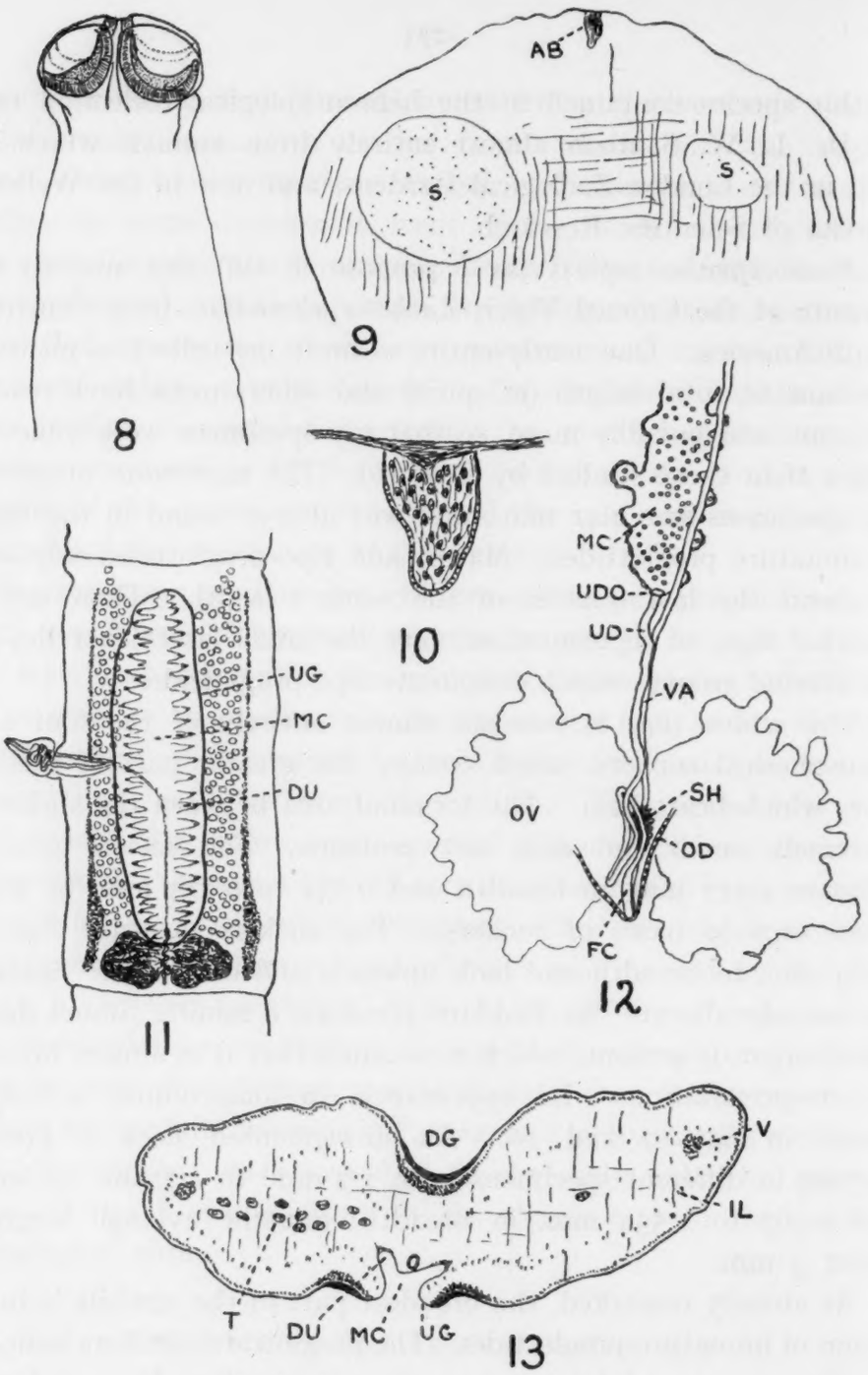
Beddard (1913c) has provided a full description of this remarkable species, and the following account only professes to supply details which Beddard has omitted and to confirm and, if possible, emphasize, his statement of the peculiar character upon which he founded his new genus *Solenotaenia*. I possess a large number of specimens

of this species contained in the helminthological collection made by Dr. L. W. Sambon almost entirely from animals which had died in the London Zoological Gardens, and now in the Wellcome Bureau of Scientific Research.

Proteocephalus viperis (as I propose to call this species) is a parasite of the Crossed Viper, *Lachesis alternatus*, from Central or South America. One nearly-entire worm in my collection measured 170 mm. in total length (in spirit) and others must have reached 200 mm. and possibly more, so that my specimens were somewhat longer than those studied by Beddard. The maximum breadth of my specimens was 2·41 mm., and was always found in the region of immature proglottides. Mature and ripe proglottides only occur in about the last quarter of the worm's length. There are no external signs of segmentation, save the small lateral notches and the uterine grooves which demarcate ripe proglottides.

The scolex (fig. 8) consists almost entirely of the four large hemispherical suckers, which occupy the greater part of the four lobes which bear them. The terminal area between the suckers is extremely small and does not protrude. The scolex measures 0·860 to 1·527 mm. in breadth and 0·531 to 0·713 mm. in length (from tops to bases of suckers). The suckers measure 0·415 to 0·664 mm. in breadth and look upwards and outwards. Spinelets are entirely absent. As Beddard remarks, a minute funnel-shaped apical organ is present, which is so small that it is almost invisible in toto-preparations. Its appearance, in longitudinal section, is shown in figs. 9 and 10. An unsegmented neck is present, varying in different specimens from 1·7 mm. to 4·7 mm. in length and 0·767 to 1·534 mm. in breadth, but the average length is about 3 mm.

As already remarked, the broadest part of the strobila is in the region of immature proglottides. The proglottides are here indicated by the presence of faint transverse segmentation lines and more posteriorly by genital rudiments and are all much broader than long, measuring from $\frac{2{\cdot}419 \text{ mm. broad}}{0{\cdot}236 \text{ mm. long}}$ and $\frac{2{\cdot}124 \text{ mm. broad}}{0{\cdot}088 \text{ mm. long}}$ to $\frac{1{\cdot}534 \text{ mm. broad}}{0{\cdot}354 \text{ mm. long}}$ (all measurements of unflattened material). Mature and ripe proglottides are not nearly so broad, the former being either



FIGS. 8 to 13. *Proteocephalus viperis.*

Fig. 8 (× 17·5). Scolex in outline.

Fig. 9 (× 56). Longitudinal section through the scolex showing the minute apical organ.

Fig. 10 (× 260). The apical organ in longitudinal section.

Fig. 11 (× 27·5). Ripe proglottis with the uterus entirely split along its whole length and empty of eggs. Note the small diverticula.

Fig. 12 (× 56). Ducts in the region of the ovary, from the dorsal view. In this proglottis the uterus has not yet split to the exterior.

Fig. 13 (× 39). Transverse section through a ripe proglottis behind the cirrus sac. Note the open uterus, the small uterine diverticula and the weak internal longitudinal muscle sheath.

AB.—apical organ ; *DG.*—dorsal groove (artefact ?) ; *DU.*—diverticula of uterus ; *FC.*—fertilization chamber ; *IL.*—internal layer (sheath) of longitudinal muscles ; *MC.*—median chamber of uterus ; *OD.*—oviduct (?) ; *OV.*—ovary ; *S.*—sucker ; *SH.*—shell-gland ; *T.*—testes ; *UD.*—uterine canal ; *UDO.*—opening of uterine canal into median chamber of uterus sac ; *UG.*—uterine groove, edge of ; *V.*—vitellaria ; *VA.*—vagina.

approximately square in shape or longer than broad, and measuring from $\frac{1{\cdot}121 \text{ mm. broad}}{0{\cdot}885 \text{ mm. long}}$ to $\frac{1{\cdot}534 \text{ mm. broad}}{1{\cdot}888 \text{ mm. long}}$ and $\frac{1{\cdot}230 \text{ mm. broad}}{2{\cdot}006 \text{ mm. long}}$, and the latter (fig. 11) always being longer than broad and measuring from $\frac{0{\cdot}885 \text{ mm. broad}}{1{\cdot}652 \text{ mm. long}}$ to $\frac{1{\cdot}357 \text{ mm. broad}}{4{\cdot}838 \text{ mm. long}}$. In my material the genital apertures are situated almost on the middle transverse line of the proglottis but not quite, being a little in front, and the cirrus aperture is usually in front of the vaginal, though the reverse condition does occur. The cirrus sac is very broad antero-posteriorly and measures in my preparations 0·298 to 0·365 mm. in length and 0·149 to 0·182 mm. in breadth, and it extends across from one quarter to one-third of the breadth of the proglottis according to the state of contraction of the latter. The cirrus sac wall is very thin, though quite well-defined, and apparently contains no muscle-fibres. The sac contains three parts of the cirrus apparatus: (*a*) an external thick-walled convoluted part which forms the outer walls of the extruded cirrus, (*b*) a long thick-walled (less thick than the first part) straight tube (the cirrus canal), and (*c*) coils of the ductus. The first two parts have attached to them ejector muscle-fibres (Beddard's 'layer of glandular cells'?). The cirrus when everted (which may equal in length half the breadth of the proglottis) is slender distally but dilated at the base which contains the ductus coils (fig. 11), and the sac, contained inside the proglottis in this condition, is relatively narrow (only 0·041 mm. broad and 0·298 mm. long in one specimen which reached a quarter of the distance across the proglottis). The sac itself then is not everted. The cirrus is not armed. The coils of the vas deferens are not very voluminous and extend to about the middle of the proglottis. The vagina opens on the same horizontal level as the cirrus and shows no marked dilatation anywhere in its course, though in contracted proglottides (not in extended) it becomes convoluted anterior to the ovary. It occasionally opens on a papilla and there is no genital atrium. The ovary in mature non-elongated proglottides is only a little more than half the breadth of the proglottis and is of the shape shown in fig. 11. There is no narrow canalicular isthmus, the follicles of the ovary extending across the middle line over a broad area. The vitellarian strands (thickened posteriorly)

have been sufficiently described by Beddard, and are of course medullary in position. The testes, as Beddard states, are very numerous, lie in two distinct fields, and measure in toto-preparations about 44 by 25 microns. I must also mention that both the vagina and the uterine canal (*vide infra*) lie on the dorsal side of the ovary (the former lying for the most part ventral to the latter), that in two of my preparations the oviducts* apparently join the vagina at the level of the hind end of the ovary (an unusual position) and that there is a recognizable shell-gland. In fig. 12 I have depicted these ducts as well as I am able to make them out, but I cannot guarantee the exact positions of the coils, nor could I detect the vitelline ducts.

Beddard has fully described the uterus and its extraordinary later development in this species, and I intend only to make one or two corrections in his account and to emphasize the features in which this uterus differs from that of other known Proteocephalidae. As Beddard says, the early development of the uterus as a median hollow stem is like that of other Proteocephalids, but he omits to lay stress upon the fact that whereas the uterus of most other Proteocephalids remains devoid of eggs until the diverticula are well developed, the uterus of '*Solenotaenia*' (like that of *P. marenzelleri* and some other snake Proteocephalids) becomes crammed with eggs while the diverticula are either entirely absent or only represented by minute irregularities of the wall (fig. 12). This fact in itself indicates that the '*Solenotaenia*' uterus is distinct from that of the majority of Proteocephalids. The next stage of development of the '*Solenotaenia*' uterus is, not the development of large diverticula, but the splitting and opening to the exterior of its entire ventral wall (the process commencing anteriorly and proceeding posteriorly, until the entire length is exposed), so that the whole cavity of the stem uterus becomes continuous with the outer world and in consequence devoid of the eggs which are at once liberated (figs. 11, 13). There is thus formed, as the final stage of development of the uterus, a deep and broad uterine groove, with smooth thickened edges, situated on the ventral side of the proglottis along nearly its entire length, i.e., from the posterior opening of the uterine duct to near

* These ducts (which are very distinct in one preparation) may possibly be the vitelline ducts, though they appear to come from the ovary and I cannot trace any connection with the vitellaria. I also admit that I cannot see these ducts in most of my preparations, nor in serial transverse sections.

the anterior end of the proglottis. This conspicuous uterine groove, as Beddard points out, is quite distinct morphologically from the apparently similar longitudinal grooves in certain Bothriocephalids and in many Proteocephalidae, since in these latter it is only a continuous depression of the body-wall which harbours the uterine pores, whereas in the former it is equivalent to the fused uterine pores themselves and represents the actual cavity of the uterus. Correlated with this formation of the uterine groove*

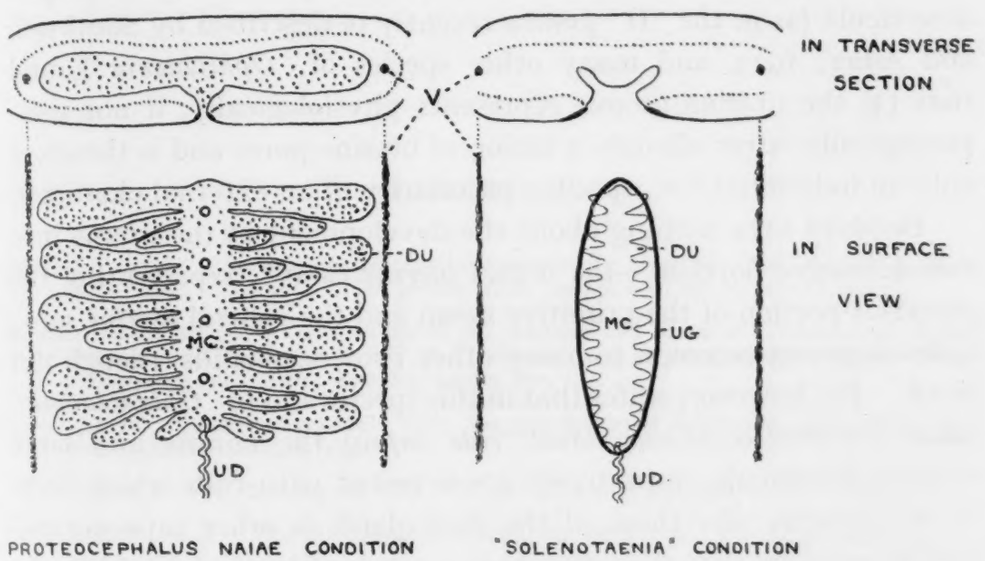


DIAGRAM to contrast the conditions of the ripe uteri of a normal Proteocephalid and of "*Solenotaenia*."

DU.—diverticula of uterus; *MC.*—median chamber of uterus; *UD.*—Uterine canal; *UG.*—uterine groove, edge of; *V.*—vitellaria.

in '*Solenotaenia*' and the early formation of the large uterine pores in allied species is the stunted development of the diverticula which are so conspicuous a feature in the fully-formed uteri of most other Proteocephalids. This difference of development of the uterus in this species, compared with the developments of the uteri of most other Proteocephalids, would afford a very much better basis for the founding of a distinct genus (cf. Lühe's characterization of the genera of the Ptychobothriinae e.g.) than the trivial scolex characters,

* In my figure 13 of a transverse section it will be observed that there is, in my material, a very distinct dorsal groove bordered by a thickened area of the subcuticula. This dorsal groove is possibly the result of local contraction, since Beddard's figures do not indicate its presence in his material.

and even the testes distribution, which have been utilized up to the present, and I would readily adopt Beddard's new genus *Solenotaenia* were it not for the facts that: (1) the stunted uterine diverticula found in '*Solenotaenia*' are also to be found in several other Ophidian Proteocephalids which do not possess the uterine groove (e.g., in *P. marenzelleri*, *P. calmettei* and '*Crepidobothrium*' *gerrardii*), and that (2) there appears to be every transition from these stunted diverticula (cf. e.g., *P. racemosa*, *P. nattereri* and the '*O*' *monnigi* recently described by Fuhrmann, 1924) up to fully-developed diverticula (as in the '*O*' *punica* recently re-described by Southwell and Adler, 1923, and many other species of '*Ophiotaenia*'), and that (3) the uterine groove represents physiologically, if not morphologically, after all only a fusion of uterine pores and is therefore only an individual, i.e., specific, peculiarity of an external character.

Beddard says nothing about the development of the uterus into two definitive portions—the dorsal *uterine canal** (representing the posterior portion of the primitive stem) and the ventral *uterine sac*—a development common to many other Proteocephalids, though not to all. He, however, states that in this species and in '*Ophidotaenia*' *naiae* (= *Proteocephalus naiae*, *vide supra*) the minute and large uterine diverticula respectively are invested with cells which seem to be 'exactly like those of the shell-gland in other tape-worms,' and he suggests that they may have a similar function, i.e., that the eggs may, in these two species, acquire their shells in the uterus instead of in the usual place. I have observed these cells investing the uterine wall in *P. viperis*, but I am not convinced of their glandular nature, and, as Beddard remarks, a shell-gland is apparently present in '*Solenotaenia*,' and is certainly present in *Proteocephalus naiae*, though Beddard failed to observe it in this latter case.

The fully-formed eggs of *Proteocephalis viperis* are fairly thick-shelled and measure about 21·7 microns in diameter, and the contained hooked embryos about 12·8 microns. In transverse section (fig. 13) the general parenchyma of the proglottis is seen to be wide-meshed and to be divided into the usual two regions of cortex and medulla by the presence of a very weakly-developed internal

* Beddard apparently figures this canal in transverse section in his text-figure 50 (p. 252) but is under the impression that it represents a convolution of the vagina. In my serial transverse sections I have followed both ducts through their entire course and have seen the uterine canal opening into the uterus sac.

layer of longitudinal muscles, a layer consisting of widely-separated small bundles, each containing only two or three fibres, and occasionally of single fibres.

The foregoing three species are provisionally placed in the genus *Proteocephalus* (syn. *Ichthyotaenia*) and not in '*Ophiotaenia*' (La Rue, 1911) or '*Crepidobothrium*' (*vide* Nybelin, 1917) for the reasons stated by me in a paper published elsewhere (Woodland. 1925).

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HUMAN TRYPANOSOMIASIS IN THE LUANGWA VALLEY, NORTHERN RHODESIA

BY

ALLAN KINGHORN

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I. PHYSICAL ASPECTS OF THE VALLEY

The Luangwa river rises in the hilly country near the junction of the Rhodesia-Nyasaland-Tanganyika Territory boundary at an altitude of from 5,500 to 5,900 feet and flows in a general south-westerly direction to its confluence with the Zambesi at Feira, 1,500 feet above sea level. On either side its valley is bounded by ranges of hills which run roughly parallel to the course of the river and which lie at distances varying from a few to as many as 40 or 50 miles from it. The country with which I am more immediately concerned in this report extends from Fundu at the southern end to the confluence of the Wira with the main river at the northern end, a distance of approximately 400 miles. Taking the average width of the valley to be 50 miles, the area thus included is at least 20,000 square miles. The Luangwa is fed by a large number of tributaries which enter it more or less at right-angles; but whereas many of those on the right side are large and permanent streams, those on the eastern bank, with one exception, flow only in the rains, and then only assume perceptible volume when carrying flood water. In general the floor of the valley is level and is covered to a very large extent by 'mopani' bush which becomes water-logged, if not actually flooded, during the rains, and in which the grass is short and of scanty growth. This mopani bush usually ends rather abruptly at some distance from the streams and is replaced by more or less open country covered by dense and luxuriant growths of grass. As it is only in these situations that the soil repays cultivation, the villages, which are small collections of from 20 to 200 inhabitants, are found strung along the courses of the larger streams. This is more particularly the case on the eastern

side of the Luangwa, as it is only in the beds of the larger streams that water can be obtained by digging in the dry season. It may be noted that at this time of the year the Luangwa itself dries up in stretches in the upper reaches.

The mean altitude of the area under discussion may be taken as about 2,300 to 2,500 feet, so that the meteorological conditions are much more tropical than in the other portions of Northern Rhodesia. At Nawalia, which is about centrally placed at an altitude of about 2,100 feet, the mean temperature varied from 67° F. in July to 87° F. in November, and the relative humidity from 31 per cent. in September and October to 78 per cent. in January. The rain-fall averages about 40 inches and is spread over the six months, November to April, though the bulk is precipitated during the first three months of the year.

II. DISTRIBUTION OF GAME AND FLY

Game is both extremely abundant and varied throughout the whole of the valley. In the rains it ranges through the whole of the area, but in the dry season, more particularly after the grass has been burnt (July to September), it tends to collect along the courses of the streams where the grazing is better than in the mopani and where alone a supply of water is assured. At this time of the year it is not uncommon to find a relatively large animal population in fairly close proximity to the villages, as many of the species, e.g., waterbuck, roan, eland and bushbuck, are fond of feeding in the old gardens. I think this point should be emphasised, as it was demonstrated at Nawalia that waterbuck and bushbuck, amongst others, were infected by *Trypanosoma rhodesiense*, the first-mentioned to the extent of between 17·8 per cent. and 25 per cent. of the total number examined. It may here be noted that in the immediate neighbourhood of some villages in Kambombo's country which have suffered severely from sleeping sickness, waterbuck are extremely plentiful. There are grounds, therefore, for regarding this species of buck with particular suspicion.

Fly. *Glossina morsitans* is to be found throughout the whole of the valley and has been observed to show much the same seasonal variation as the game, i.e., in the dry season it is abundant near

the streams and hence near the villages, but after the rains have commenced tends to become more uniformly scattered over the country as a whole. This peculiarity has been observed in other parts of Africa. Owing to the fact that the villages are, as a rule, built in clearings and are surrounded by gardens, it is unusual for the fly to invade them, though an occasional specimen may follow the natives in. If so, it soon disappears again.

III. HISTORY OF SLEEPING SICKNESS

Attention was first drawn to the occurrence of human trypanosomiasis in the valley by the diagnosis of the disease in several Europeans about the year 1909. Investigations commenced then and carried out in the succeeding years showed that it was scattered over the whole of the area though the number of cases found was comparatively small. They demonstrated further that the infection displayed no tendency to assume epidemic proportions. Writing at the end of 1912, the Principal Medical Officer stated: 'In the light of recent knowledge as to the presence of the necessary factors and apparently very suitable conditions for the production of an epidemic, it is difficult to understand why after four years there should be no evidence that such is likely to occur. It can be most reasonably concluded that there is some unknown but necessary factor wanting or some inhibitory influence present, i.e., that the disease is an old one and that there may be a certain immunity present which is limiting its spread.' This is in strict accordance with the native evidence, for those I have questioned have always consistently maintained that they have always known of the existence of the disease as far back as their memories carry, in some cases a matter of seventy years or so. They state that it cropped up only as isolated cases which were drastically disposed of in some localities. Amongst the Bawisa and Bansenga the terms 'Chilotera' and 'Nyamakazi' are used to denote the symptom-complex of fever, oedemata of the extremities, protuberant abdomen, diarrhoea and emaciation, all commonly observed in sleeping sickness, though, of course, they do not associate the disease with tsetse flies. My own belief coincides with that of the natives, that the disease is an old one and not of recent introduction.

IV. INCIDENCE OF THE DISEASE

The observations with which I am now about to deal are all, with few exceptions, derived from data collected by me during 1913 and the early part of 1914, and after the conclusion of the war, from 1920 onwards. In this work I have relied on gland palpation and puncture as the means of diagnosis and only departed from it in exceptional cases and for special reasons, e.g., where a native's illness appeared to be due clinically to sleeping sickness but no palpable glands were found. Under these conditions, I claim that the various sets of figures may be properly compared and that they give a real indication of the relative incidence of the disease in the different years and in the same localities. I do not claim, however, that they indicate the absolute incidence of the disease. While I am of the opinion that gland palpation and puncture is the quickest and only feasible method of examining large bodies of natives, and that the great majority of cases will be found by its employment, I admit that some cases, particularly those in the very early stages of the infection, before the lymphatic glands have hypertrophied and in which no other symptoms are present, will be missed.

As mentioned earlier, the disease is very widely distributed and has been found in the Petauke, Serenje, Fort Jameson, Lundazi, Mpika and Chinsali portions of the valley. Further it is, as a general rule, comparatively rare, as the following figures will demonstrate :—

Year examined	District	Natives examined	Cases	% infected
1913	Mpika	2,613	2	0·08
	Lundazi	13,100	9	0·07
	Chinsali	1,465	4	0·26
1914	Serenje	1,981	2	0·10
	Petauke	3,654	2	0·05
		23,113	19	0 08
1921	Lundazi, Part	3,723	1	0·03
	Mpika „	1,311	0	0·00
	Chinsali „	913	1	0·11
		5,947	2	0·03

As illustrating the general tendency for the disease to remain stationary over a period of years the following figures may be quoted. In every instance they are for the same villages in the respective years.

District	Year	Natives examined	Cases	Year	Natives examined	Cases
Lundazi	1913	2,812	0	1921	3,530	1
Mpika		1,233	0		1,261	0
Chinsali		776	3		913	1

A further example may be quoted from Dr. May's investigations in one particular area in the Petauke Sub-District where, in the three successive years 1910, 1911, and 1912 respectively, 7, 7, and 3 cases were found.

It is difficult, in the absence of a complete census and the registration of births and deaths, to estimate not alone the general death rate amongst these natives but also that more specifically due to sleeping sickness. The following calculations, however, may be given. At the end of 1912, Dr. May computed the adult death rate in a portion of the Petauke Sub-District to be 28 per 1,000 and the incidence of sleeping sickness to be 8 per 1,000, also amongst the adults only. In 1913 I made similar enquiries as carefully as was possible in the valley portion of the Mpika Sub-District and estimated the general death rate for the year 1912-1913, exclusive of accidents, to be 23·7 per 1,000, the adult rate to be 47·7 per 1,000 and the incidence of sleeping sickness to be 3 per 1,000 of the whole population seen. Speaking of the valley generally, I should be inclined to say that under ordinary circumstances the incidence of the disease is not in excess of 3 to 4 per 1,000 of the total population per annum, though of course it may be exceeded temporarily in those localities in which exacerbations of the infection occur (for example, the estimated death rate for 1920-21 of 25 per 1,000 in the countries of chiefs Tembwe and Kambombo).

A very striking feature of the infection is the extraordinarily sporadic manner in which it is found. Not only are the cases found in villages widely separated but they are also usually found occurring singly, and cases in the same village may be separated by an interval of years. Examples of this are given in the following tables, and it should be noted that approximately the same number of natives were examined on the various occasions.

Village	Wallace, 1912	Kinghorn, 1913
Mumamba	1	0
Daroba	1	0
Chuni	0	1
Mkasanga	0	1
Chombero	0	1
Kundawamawe	0	1
Temba	0	1
Chinyondo	0	1
Luchenga	0	1
Kampuzunga	0	1
Mulumgu	0	1

Village	1920	1921	1922	1924	1925
Tembwe Virizi	0	2	1	0	0
Katangalika	0	1	0	0	0
Ng'anjo	1	0	0	0	0
Mwimba	0	1	0	0	0
Kajumba...	1	1	0	1	0
Kambombo	0	1	0	0	0
Chizonde...	0	1	0	0	0
Kambwiri	1	0	0	0	0
Kazembe...	0	0	1	0	0
Dungulungu	0	1	0	0	0
Chama	1	1	0	0	0
Kawanda...	1	1	1	0	0
Kapalakonje	1	0	0	0	0
Chitimbe...	1	1	1	0	0
Nyika	2	1	0	0	0
Luambo	2	0	1	0	0
Chileta	0	0	1	0	0
Hunga	1	2	0	0	0
Buli	1	0	0	0	0
Chitukula	1	0	0	0	0
Chiruarua	2	0	0	0	0
Zowole	1	0	0	0	0
Mtonya	1	0	0	0	0
Mkunguwe	1	0	0	0	0
Luchenga	0	0	2	0	1
Kapotwe	0	1	0	0	0
Makondola	1	0	2	0	0
Kakuni	0	0	2	0	0
Marunga	1	0	0	0	0

How is this peculiar distribution and incidence to be explained? As is well known, some observers, chiefly the Germans, maintain that there are two distinct trypanosomes, *brucei* and *rhodesiense*, existing side by side in tropical Africa, which are indistinguishable except for the fact that *T. rhodesiense* is capable of infecting man while *T. brucei* is not, but is restricted to game and stock. The other school, chiefly British, maintain that the two trypanosomes in question are identical; that it is essentially a parasite of game; and that man is ordinarily resistant to infection, though this may occur. Exactly what conditions are necessary before man does become infected are uncertain. The chief arguments of those who favour the non-identity hypothesis are: (1) Dr. Taute's experiments, and (2) the geographical argument, that in many localities where *T. brucei* is found cases of sleeping sickness have never been diagnosed. With reference to the first of these, I think the experiments may be held quite permissibly to prove the truth of the contention that man is naturally resistant to infection by *T. brucei vel rhodesiense* and that, in any event, they are not sufficiently extensive to prove indisputably the truth of the negative statement that the human and game trypanosomes are not identical. As regards the geographical argument, I am not aware that the localities usually cited have been thoroughly and repeatedly examined over a period of years, and that, at the same time, such important factors as the abundance of fly and game, the percentages of both harbouring *T. brucei, sensu strictu,* and the closeness of contact existing between them and the native population have been taken into consideration. Without departing from the confines of Northern Rhodesia, however, it appears to me to be a difficult matter to explain, on the assumption that human cases of the disease are invariably due to a specific human as distinct from the ordinary game trypanosome, the occurrence of one European and two or three native cases in the western part of the Serenje sub-district with an interval of years between them, more particularly as the examinations of the suspected area carried out by Dr. Masters prior to 1912, by Dr. Ellacombe in 1912, and again by Dr. Powell in 1920, gave negative results as far as the indigenous population was concerned. And further, it is peculiar that in the other area in this country in which the disease has been found

to occur much as it does in the Luangwa valley the same conditions of an abundance of game and fly co-exist in close contact with the natives. I refer to the focus in the south-western corner of the Ndola sub-district. Prof. Kleine, one of the protagonists of the non-identity theory, admitted in conversation that the local game was susceptible to infection by *T. rhodesiense, sensu strictu*, and this being the case it follows that it would only be a matter of time until this parasite was widely distributed amongst the fauna of the valley and one would then expect to find human cases to be both more numerous and more uniformly spread over the country as a whole. As pointed out above, there is a concentration of both the game and the fly in the vicinity of the villages in the dry season, and while a certain amount of evidence exists to show that the risks of infection are then greater it is not extensive enough to permit of any dogmatic statement on the point. I believe, personally, that the sporadic appearance and erratic distribution of the infection as it is generally seen and has been seen since 1909, with no tendency to assume epidemic proportions, is more satisfactorily explained by the theory that the human and game trypanosomes are one and the same parasite and that man is ordinarily resistant to infection by it than by the theory that the human and game trypanosomes are distinct entities.

While the normal incidence of the infection is as shown above, exacerbations have been observed from time to time in localized areas of the valley, but these, after the lapse of a few years, have always ended spontaneously and the disease has then reverted to what may be termed its equilibrium. The most pronounced of these has been the one which started early in 1918, in the contiguous territories of the three chiefs Chikwa, Tembwe and Kambombo towards the Northern end of the Lundazi sub-district. Of the three, Kambombo suffered most. Thus writing in May, 1920, the Native Commissioner, Lundazi, reported that since the commencement of this outbreak some 131 deaths had occurred to date from what appeared to be sleeping sickness, amongst an adult population estimated at 1,455 on March 31st, 1920, in the villages under that chief. It may be noted that at the end of 1917, or beginning of 1918, plague appeared in this same area and was not stamped out until 1919. This coincidence was probably quite fortuitous except that many of the

villages were burnt and the natives forced to build temporary quarters in the bush. This, of course, brought them into closer and more continuous contact with fly than is ordinarily the rule, and to some extent an increase in the number of cases of sleeping sickness may be ascribed to this cause, but it cannot have been of general application, as those villages which suffered most severely from sleeping sickness were not destroyed. In 1920, and the succeeding years, I examined and re-examined the natives in this particular area with the following results :—

Year	Natives examined	Cases	% infection
1920	5,756	25	0·43
1921	5,634	18	0·32
1922	5,317	9	0·17
1924	4,347	1	0·02
1925	4,301	2	0·04
Compare 1913	5,122	3	0·06

Or if we consider the three sets of natives separately we have the following percentages of infection in the various years :—

Year	Percentage Infection		
	Chikwa	Tembwe	Kambombo
1920	0·25	0·73	0·36
1921	0·15	0·27	0·57
1922	0·22	0·12	0·15
1924	0·00	0·00	0·06
1925	0·07	0·00	0·06

It should be noted that these percentages only represent the incidence of the disease at the actual time of examination, and, while under ' normal ' conditions they might afford an approximate idea of its frequency, they are much too low for ' Epidemic ' conditions. Thus in 1921, by tracing the causes of deaths in the interval between that and the former visit, I estimated that the then rate of infection was about 2·5 per cent. of the whole population per annum. I am not fully satisfied that the 1924 figures give anything like a true indication of the percentage of infection in that year, as I saw about a thousand fewer natives than in former trips, and amongst those who evaded examination cases may have existed ; but it may be noted that the deaths between 1922 and 1924, which could be attributed to sleeping sickness were comparatively few ; hence it seems reasonable to conclude that this localized ' semi-epidemic ' is behaving like the others and that the infection is becoming stabilised again. That this conclusion is essentially true is borne out by the results of the trip I have just finished through this area. As will be seen above, 4,301 natives were seen and 2 cases of the disease diagnosed, a percentage of 0·04. In the six months which elapsed between my 1924 and 1925 trips some nine deaths, which might possibly have been due to sleeping sickness, occurred, so that it would appear that the annual incidence of the disease in this particular area is now not in excess of 5 per 1,000 of the total population.

I am of the opinion that these epidemics are due largely to the superimposition of a man-fly-man cycle of transmission of the parasite on the more ordinary game-fly-man cycle and the occurrence of this is often hastened by some of the habits of these natives. For instance, cases may be carried from one village to another owing to the custom of returning to the original home when a native falls sick. Again, during the rains the villages split up, each family living in the middle of its gardens in order to protect them from the depredations of monkeys and elephants, and cases of infection in man and wife and parent and child have been observed under these conditions. Further, the habit of growing crops between the huts in the villages themselves increases the liability of their invasion by fly with the consequent danger of their becoming infected should a case of sleeping sickness exist.

V. THE DISEASE IN HUMAN BEINGS

The incubation period is short, probably between one and two weeks. At first the only symptom is fever with its concomitants, followed by enlargement of the lymphatic glands, particularly in the basal portion of the posterior neck triangles, progressive emaciation, oedemata of the extremities and face, protuberant abdomen, diarrhoea, anaemia, muscular tremors, inco-ordination, mental hebetude and somnolence deepening into coma. In untreated cases, death is the inevitable result and the whole duration of the infection is short, on the average from about three to six months. An occasional case may live for ten or twelve months, though this is very exceptional, and only one, in my experience, has ever exceeded this period.

This native, Chimwila, was found to be infected on the 18th November, 1920, and then gave a history of having been ill only one month, complaining of headache. This was probably an underestimate as his neck glands were markedly enlarged. He was seen again on the 9th May, 1921, and appeared to be in perfect health saying himself that he was not sick. His neck glands were now smaller though the juice still contained parasites. His condition remained the same on the 11th March, 1922, and trypanosomes were still found in the gland juice. He was then sent to Prof. Kleine for treatment with Bayer 205, i.e., sixteen months after he had been found to be infected and probably eighteen at least after he contracted the disease. Prof. Kleine states in one of his reports that Chimwila appeared to be physically in good health on his arrival but that some mental dullness was noticed. After receiving treatment he returned to his village later in 1922 and was apparently quite well when last seen in March, 1925. No glands were palpable and no parasites were found in the blood.

The disease is very decidedly one of adolescence and adult life. I have never seen a case in a young child, and only comparatively rarely under the age of about fifteen. This is well brought out in the following tables from two independent sources, and in view of the great difficulty of estimating at all accurately the ages of natives and the part the personal equation must play in such estimations, the general agreement of the two sets of figures is very striking.

Age	Kinghorn		Fischer	
	Cases	%	Cases	%
6-15	5	6·66	1	3·57
16-25	20	26·66	9	32·14
26-35	27	36·00	11	39·28
36-45	17	26·66	5	17·85
46-55	5	6·66	1	3·57
Over 55	1	1·33	1	3·57
	75	99·97	28	99·98

It will be noticed from these tables that there is a steadily increasing susceptibility to infection from the earlier ages to about the 35th year and that thereafter the decline in susceptibility is as equally and steadily marked; further, that from 85 to 89 per cent. of all the cases occur in the age group 16 to 45. These remarks apply with equal force to the two sexes considered separately, as shown below.

Age	Males		Females	
	Cases	%	Cases	%
6-15	4	8·88	1	3·33
16-25	11	24·44	9	30·00
26-35	15	33·33	12	40·00
36-45	12	26·66	5	16·66
46-55	3	6·66	2	6·66
Over 55	0	0·00	1	3·33
	45	99·97	30	99·98

This table also brings out the fact that the disease is commoner in men than in women, the proportion being as 3 : 2, and this is corroborated by an analysis of Dr. Fischer's cases, which gives a proportion of $3\frac{1}{2}$ males to 2 females. Probably the greater number of male cases may be accounted for on the basis that the men, on the whole, spend more time in the bush, hunting, collecting honey, poles for building, reeds and other material for mat and basket-making, and so on, than the women do in their daily trips to collect firewood. It is more difficult to explain why, approximately after the age of puberty is passed, the susceptibility to infection should increase so perceptibly and increase in an ascending curve to the age of thirty-five and then decrease steadily. Babies and very young children are always carried by their mothers in a calico or sling and are thus fairly effectively protected, but the older children who also usually accompany their mothers wherever they go are naked and unprotected. The older children, aged from ten to twelve onwards, are, at best, very scantily dressed and have to assist their parents in the various duties outlined above. They are thus frequently exposed to the risk of infection and yet it is only very rarely that this occurs. It may be argued that the discrepancies in the age-incidence of the disease are more apparent than real and that if the actual age distribution of the whole population could be plotted, these would become obvious, but in the absence of an accurate census this is impossible. I am, however, inclined to doubt this. Certainly as between children and adults it does not apply, as it may be said generally that the number of children in a village will about equal the number of adults. Thus an analysis of the two groups in the villages under Chikwa, Tembwe and Kambombo, gives 2,741 children to 3,015 adults. I am convinced that the varying rates of infection amongst the different age-groups cannot be explained wholly and satisfactorily on the assumption that they are in direct ratio to the risks of infection run by the respective groups, though it is difficult to suggest other factors which may influence the occurrence of the disease in the light of our present knowledge. It may here be pointed out that hunger with its consequent lowering of vitality does not increase susceptibility to infection. In this area the 1923-24 crops were bad, with the result that, towards the end of 1924, acute hunger,

amounting in some villages to actual starvation, became apparent, and several deaths from this cause were reported. The results were particularly noticeable in Chikwa's country, where all of the natives showed emaciation varying from slight to extreme, yet only one case of sleeping sickness was found, and only five deaths from what may have been sleeping sickness were reported amongst 1,400 natives.

There is no evidence that acquired immunity to the infection is found in man though, as stated above, I am of the opinion that he is naturally very resistant to it.

VI. TREATMENT WITH 'BAYER 205'

Early in 1922, a commission composed of Prof. Kleine and Dr. Fischer came out to this country to try the effects of the drug known as 'Bayer 205' on human trypanosomiasis and a camp was established in the Luangwa valley at Ndombo, about thirty-five miles east of Mpika. In all, 38 cases were treated, and of these one man went later to Southern Rhodesia and has not been traced, five are still alive, and the remaining 32 are dead. Five of the deaths occurred at the Commission's camp from intercurrent affections and the other 27 in the villages at varying periods after the parents had returned there on leaving Ndombo in October, 1922. The following is the complete list :—

Name	Sex	Age	Date of death	Duration of life after treatment	Remarks
1. Pitala	M	25	February, 1924	16 months	
2. Yatula	F	30	—	—	Alive
3. Chimwila	M	35	—	—	Alive
4. Chizilicho	M	35	March, 1924	17 months	
5. Isake	M	15	July, 1923	9 ,,	
6. Chilikupata	M	25	April, 1924	17 ,,	
7. Mofati...	M	35	?	?	Untraced
8. Lamek	M	16	—	—	Alive
9. Samuel	M	16	February, 1924	16 months	
10. Tadeyu	M	27	February, 1924	16 months	

Name	Sex	Age	Date of death	Duration of life after treatment	Remarks
11. Kajawa	M	38	—	—	Alive
12. Vioka	F	22	October, 1923	12 months	
13. Mateyo	M	35	February, 1923	4 months	
14. Chifundulwa	M	60	—	—	Alive
15. Lasalu...	M	25	February, 1924	16 months	
16. Vilauli	M	38	July, 1924	19 months	
17. Malita	F	22	November, 1923	13 „	
18. Mwipi	M	12	December, 1923	14 „	
19. Murere	F	45	October, 1923	12 „	
20. Nderema	F	40	October, 1923	12 „	
21. Ntanda	F	27	October, 1923	12 „	
22. Yumba	F	45	November, 1923	13 „	
23. Ndabeya	F	54	April, 1923	6 „	
24. Mage	F	26	February, 1924	16 „	
25. Marata	M	28	July, 1923	9 „	
26. Mapulanga	M	35	February, 1923	5 „	
27. Kabrieni	M	18	November, 1923	13 „	
28. Chikoti	M	35	October, 1923	12 „	
29. Kamchepa	F	32	October, 1923	12 „	
30. Zakeyo...	M	35	October, 1923	12 „	
31. Wayilipa	F	35	October, 1923	12 „	
32. Sabeta	F	20	October, 1923		Died on road home
33. Thomas	M	17	February, 1924	16 months	
34. Thomas	M				
35. Ngoza	F				
36. Chiweza	M		died at Ndomba from intercurrent disease		
37. Tepatepa	M				
38. Puntayila	M				

As will be noticed, the cases were of both sexes and of all ages and it may be added that they were in various stages of the disease from early to late. With regard to the cases which died in the villages, the native evidence is that at varying periods after they had returned they started to become ill again and presented the ordinary symptoms of sleeping sickness, e.g., progressive emaciation, oedemata, and so on. This applies equally to the eight cases which died in October, 1923, but there is a possibility that in these death was hastened by influenza of which an epidemic swept through the valley in August and September of that year. This reappearance of symptoms must be regarded as due to relapses of the original, and not to fresh infection. In the treatment of sleeping sickness the greatest drawback to success is the difficulty of attacking effectively the parasites in the cerebro-spinal fluid, and evidence of this with 'Bayer 205' is found in the reports of the German Commission. Thus it is stated that of twenty-one patients examined by lumbar puncture before their discharge from the Ndombo Camp in October, 1922, eight were found to harbour trypanosomes. Under such circumstances it would only be a matter of time until the trypanosomes re-invaded the blood stream and set up symptoms of the disease. The only definite result claimed by the Commission for 'Bayer 205' is that by its use it is possible to sterilise the blood for a long period even in those cases which are not clinically cured; and while this claim seems to be established I question, in view of the demonstrated fact that the bulk of the cases did ultimately relapse, whether there are any substantial grounds for stating that 'if in districts infected with sleeping sickness all suspected natives receive treatment . . . the source of infection for the tsetses will gradually disappear and in time the disease must die.' It is assumed that man is the only reservoir and that the game is negligible. This I cannot admit.

The routine method of treatment adopted by the Commission was three subcutaneous or intravenous injections of 1·2 gm. in normal saline at intervals of ten and eighteen days, though those cases in which the parasites persisted in the blood and cerebro-spinal fluid received a fourth and fifth injection. It is apparent, therefore, that a dosage of from 3·6 to 6 grammes is insufficient to cure the majority of cases of *T. rhodesiense* infections in natives. Better

results might, of course, follow the adoption of an increased dosage up to 10 or 12 grammes as has been advised by some experienced workers, but at the price of 6/6 a gramme it is questionable whether the expenditure of £3/5/– to £4 per head on drugs alone would be legitimate, in the light of our present knowledge, on any very wide scale, more particularly as reports from the Congo would indicate that in tryparsamide we possess an equally, if not more, efficacious drug which possesses the advantage of being cheaper. It is of interest to note that in these reports it is said that the results of treatment there with 'Bayer 205' had been 'frankly disappointing.'

I saw and examined the five cases which are still alive in August, 1924, and again last month. All of them appeared to be in perfect health, presented no signs or symptoms of sleeping sickness, and showed no parasites in the blood stream. It was not possible to perform lumbar puncture. I believe, therefore, that these natives may now be regarded as being definite cures. Disregarding the five cases which died from intercurrent disease and the one which has not been traced, this represents a percentage of 15·6 cured. In view of the fact that all previous methods of treatment for the Rhodesian type of the disease have been failures, this must be admitted to be a considerable advance, but these results do not justify the very optimistic claims which are still being made for this drug.

VII. PROPHYLAXIS

It is a definite fact, which must be recognised, that no active assistance can be expected from these natives for any measures designed either to combat a specific infection, or generally to improve the sanitation of the villages. For some years movement into and out of the valley was prohibited, and even though the sleeping sickness regulations have fallen into comparative desuetude very few Europeans travel there now. Thus to a marked degree the natives have remained under the influence of their ancient tribal beliefs and treat European ideas of the etiology of disease and the methods to be adopted in treating them with undoubted, if unexpressed, disbelief. The general attitude, therefore, becomes one of passive resistance which can only be overcome by a certain

amount of compulsion, and when force has to be employed the results are usually unsatisfactory. Not only does the native revert to his own ideas and habits as soon as he thinks he can safely do so, but there is also a tendency to evade actively the application of disagreeable rules and regulations by running away and hiding as soon as an official appears in his district. Of this I have had personal experience. This becomes more pronounced when the results of European regulations and treatment are unsatisfactory, as it must be admitted they have so far been with particular reference to sleeping sickness. My experience is that the few cures are overlooked and attention is concentrated on the failures, and that indeed the natives really believe that the deaths have been caused by the use of hypodermic needles. In time, and with the increase of education, this general attitude may be modified but it will necessarily be a very slow process, and in the interval it is not apparent that much can be done. Something might be done to hasten this, by arrangement with the various missions having schools in the valley, if the teachers were given instruction in the essential facts of the etiology of sleeping sickness and other infections, told to explain these facts to their classes at frequent intervals, and to urge patients to go to hospital for treatment. No immediate results could be expected in view of the ingrained instinct of the native to return to his home as soon as he falls sick, and in view of the widespread aversion to going into hospital. I think, however, it would be a step in the right direction.

In view of the facts, which I think have been brought out earlier, that sleeping sickness ordinarily is one of the less important causes of death in the valley and that in general it occurs only in widely-scattered, sporadic cases, I am inclined to doubt whether it is incumbent on the Government to provide special facilities for treatment beyond those which already exist in the various hospitals. When the enormous area of the valley is recalled it is obvious that to do so would entail, if success is to be obtained, the appointment of at least three special medical officers with hospital and staffs, and the expense of this would, of course, be very large. Success could only be anticipated if the theory is true that *T. rhodesiense* is a parasite of human beings alone, that man is the sole reservoir and that the game plays no part in the perpetuation of the infection.

If so, then it appears to me that the obligation to deal with the disease by special measures is greatly strengthened. If, however, it is correct that the game and human parasites are identical, or alternatively that the game may act as a reservoir for a specific human trypanosome, then success in combating sleeping sickness can only be assured by simultaneously segregating and treating all the human cases of the disease and killing off the whole animal fauna. This is not a practicable proposition in the valley.

In the event of the occurrence of one of the localized epidemics it would, I think, be advisable to institute local treatment, as I believe that in these man does play a part as a direct reservoir of infection and it would be a matter of some importance to break the man-fly-man cycle.

I also think that it would be advisable to keep on the statute book the Sleeping Sickness Regulations, not with any idea of interfering with the natives, but chiefly for the power they confer of regulating the movements of Europeans. If all the regulations were rescinded, there would probably be an influx of professional hunters in pursuit of elephant, and the possible occurrence of cases amongst them might entail an unnecessary expense on the Government.

Lundazi, N. Rhodesia,
April 10, 1925.

THE DESTRUCTION OF ASCARIS EGGS

BY

SEIJI OGATA

PATHOLOGICAL DEPARTMENT, OSAKA MEDICAL COLLEGE, JAPAN

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Most investigators are agreed that Ascaris eggs have strong powers of resistance to chemical fluids of various kinds. I also have tested the resistance of Ascaris eggs containing embryos by soaking them in various disinfectants, acid or alkaline, and have found that in every experiment they retained for many days the ability to infect.

I found, however, that Ascaris eggs were easily destroyed by heat. In September, 1921, I poured hot water at a temperature of 100° C. over Ascaris eggs collected from human dejecta and found that the eggs lost their power of development. In November of the same year I noticed that Ascaris eggs containing embryos, after hot water had been poured on them, lost the ability to infect.

Having ascertained that Ascaris eggs were instantly destroyed by water at 100° C., I attempted to discover how long they would remain alive in water at varying degrees of temperature. These tests, however, failed on account of the difficulty of finding any effective means of immersing in hot water Ascaris eggs dispersed among human dejecta. Again, if eggs mingled with human dejecta are dispersed in boiling water, it is not always possible to get them out at once.

At length, a method was discovered which seemed to me entirely effective. The following experiments were than carried out.

Method: In testing the resistance of Ascaris eggs to heat, I employed matches in order to prevent the dispersal of the eggs. The human dejecta were washed in water and the sediment obtained by centrifuging was smeared on the matches to half their length, and left to dry. Then each match, held with the forceps, was soaked for the desired period in hot water at varying temperatures. They were then put into cold water to cool. The matches were then subjected to observation.

The observations were carried out in three ways, (1) staining; (2) development; (3) infection.

(1) I use Sudan III staining for the purpose of distinguishing between live and dead Ascaris eggs. Presuming that fatty

degeneration might possibly occur in the liver of a patient infected with Ascaris, we applied Sudan III staining to the liver of an animal which was experimentally infected with Ascaris. We found that Ascaris larvae in the liver were distinctly stained by Sudan III staining, and that living larvae separated from the liver or lungs could be similarly stained. The red-stained granules of 'fat-corpuscles,' as I provisionally call them, gradually decrease in size as the size of the larva increases at each stage of its development.

With the object of making clear the true nature of the ' fat-corpuscles ' of the Ascaris larva, I tried to ascertain if there were any substance in Ascaris eggs which could be stained with Sudan III. It was necessary to make sections for the staining of the eggs. The Ascaris eggs, collected from human dejecta, were embedded in gelatine and cut into thin sections with the freezing microtome. They were then stained with Sudan III. By this means the staining of eggs was obtained, but not of the albuminous membrane. I further noticed that fat-corpuscles were abundant in the eggs.

When I applied Sudan staining to Ascaris eggs collected from human dejecta, I found that unfertilised eggs were stained with red granular spots, while healthy fertilised eggs were not stained at all. Therefore, presuming that Ascaris eggs might be made stainable by killing them, I applied Sudan III staining to eggs killed by boiling water. As I expected, all the eggs were seen under the microscope with fine red colouring.

From the results of my repeated experiments, I conclude that the fat-corpuscles are produced in the blastomeres as the egg-cells grow and segment, and that gradually these fat-corpuscles gather much more in the hypoblastic than in the epiblastic cells ; thus very young U-shaped embryos are full of fat-corpuscles from head to tail ; but these fat-corpuscles are ranged along the intestine of the worms as they grow. We consider, therefore, that the above-mentioned fat-corpuscles may be the yolk.

Having found, therefore, that (*a*) healthy fertilised eggs are unstainable with Sudan III, that (*b*) unfertilised eggs are stainable, and that (*c*) fertilised eggs become stainable with that dye if hot water is poured over them, we used Sudan III for the study of Ascaris eggs after immersion in hot water at temperatures varying from 40° C. to boiling point, and for periods varying from one second

to an hour. We found that Ascaris eggs become stainable with Sudan III after being immersed for one second in water at over 75° C., almost all become stainable after being immersed for ten seconds in water at 70° C., while in water at 65° C. they had to be immersed for over ten minutes before becoming stainable. In water at temperatures lower than 60° C. over one hour's immersion is not sufficient to render them stainable.

The above experiment was repeated twenty times with material from the same patient and from twenty-one others, but the results showed no great difference.

(2) In order to prove that Ascaris eggs which have become stainable are really dead, we cultivated, in 4 per cent. formalin solution, Ascaris eggs which had been immersed in water at varying temperatures for varying periods of time as in Experiment 1, and examined their developmental condition at the end of stated periods. This was repeated some thirty times with material from the same patient and from many others, but all the results were practically the same, namely, that all the eggs lost their power of development after being immersed in water at over 70° C. for one second; a few retained it after being dipped in water at 65° C. for one second; embryos developed from all eggs immersed for three seconds in water at 60° C., for 40 seconds in water at 55° C., and for thirty minutes in water at 50° C.; all eggs would develop into embryos after being soaked for one hour in water at temperatures lower than 45° C.

The difference between the results of Experiments 1 and 2 is noteworthy.

Ascaris eggs which have lost their power of development can be divided into two groups, one stainable with Sudan III, the other unstainable with that dye. The former show a morphological change (i.e., fatty degeneration and distortion of the eggs) after immersion in hot water; the latter do not differ from healthy eggs, and yet are in a state of suspended development. This condition of suspended development may continue for as long as twenty days or even longer, but if left alone for long they are likely to perish gradually.

Ascaris eggs are instantly killed by very hot water; by water at lower temperatures they are merely deprived of their ability to develop; and after being immersed in water at temperatures below 45° C. the eggs develop in the usual manner.

(3) As a result of Experiments 1 and 2 I could definitely fix the temperature at which hot water will destroy Ascaris eggs containing embryos capable of infection.

I cultivated for a month, in 4 per cent. formalin solution, Ascaris eggs collected from human dejecta. When matured, I placed them on matches and left them to dry until the following day. I then soaked these matches in hot water at various temperatures and fed mice with the eggs. After three days I examined the mice with a view to ascertaining whether liver, heart, or lungs were infected. I repeated this experiment many times with 515 mice and arrived finally at the following results.

The mature Ascaris eggs, cultivated in 4 per cent. formalin solution, lose their infecting power after remaining for one second in water at 70°.C. or more ; almost all lose it after remaining for one second in water at 65° C., for five seconds in water at 60° C., for forty seconds in water at 55° C., or for fifteen minutes in water at 50° C. Even after remaining for one hour, however, in water at temperatures below 45° C. the eggs did not lose their power to infect.

SUMMARY

A minute examination of the power of resistance to heat of Ascaris eggs by means of the above experiments has led to the conclusion that the method of destruction of Ascaris eggs by boiling water can be effected also with hot water at lower temperatures. Generally speaking, the ideal to be aimed at in disinfection is simplicity of method and rapidity of action.

To prevent Ascaris infection it is safest to kill the eggs by immersion in hot water at the temperature at which the egg content changes morphologically and becomes stainable with Sudan III ; though the power of development and the power to infect may be destroyed by immersion in water at over 70° C. for one second, at 65° C. for two seconds, at 60° C. for five seconds, at 55° C. for fifty seconds and at 50° C. for forty-five minutes.

In conclusion, I wish to acknowledge my great indebtedness to Dr. S. Yoshida who kindly superintended my work and gave me all facilities for completing the present paper.

AN EXPERIMENTAL STUDY ON THE DEVELOPMENT OF THE DWARF TAPEWORM *(HYMENOLEPIS NANA)*

BY

YOSHIHISA SAHEKI

RED CROSS HOSPITAL, NAGANO, JAPAN

(Pediatrics Laboratory of Kanazawa Medical College, Kanazawa, Japan)

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The course of infection and the development of the dwarf tapeworm have been much studied and discussed, especially as to whether this species has any intermediate host or not, but as yet no definite conclusion has been reached. I have studied this subject extensively, both in animals and in man.

From my experiments it appears that the eggs of this tapeworm, unlike those of others, can hatch and develop without any intermediate host.

ON THE DEVELOPMENT OF THE DWARF TAPEWORM IN ITS HOSTS

1. *From five hours to twenty-four hours after feeding.*

On examining mice which had swallowed dwarf tapeworm eggs five or six hours previously, I observed in their small intestines six-hooked larvae, all of which had emerged from the egg-shells. After ten hours the mice had larvae which had already entered into the villi in the upper part of the small intestine and from hour to hour, up to twenty to twenty-four hours, larvae penetrated the villi. These larvae were oval or round in shape, their surface was granulated and they had six hooks near one end.

2. *Three days after feeding.*

I found many larvae in the villi; their shape was the same as noticed above but they had increased in size. Most of them were somewhat pear-shaped. In the centre of their bodies the granules

became somewhat coarse and I observed some small calcareous corpuscles which reflected the light strongly, but as yet the larvae were not encysted.

3. *Four days after feeding.*

The six-hooked larvae, which had entered the villi, had increased much in size and became bean-shaped encysted larvae (Cysticercus), the space between the body of the larva and the cyst being filled with granular material. Some, still with six hooks at one end of the cyst, were moving in the villi.

In the centre of the body of the larvae was a ring of regularly arranged wedge-shaped hooks, which reflected the light strongly; but the roots of the hooks were not yet separated and the differentiation of the suckers was not clearly defined; however, by carefully adjusting the light, I could see some encysted larvae with two suckers in front and some with only one; and among the radiating fibres running from the caudal extremity of the encysted larva to the cyst, could be found irregularly-shaped calcareous corpuscles, some large, some small.

4. *Five days after feeding.*

Well developed encysted larvae had emerged from their cysts, left the villi, descended to the lower part of the small intestine and were actively moving about, extending and contracting themselves; some were in process of emergence from their cysts and some had already emerged, still having parts of the cysts attached at the rear. In these larvae I could see four suckers; a single ring of hooks, an excretory tube, running from the rear of the rostellum to the caudal extremity, and some irregularly-shaped calcareous corpuscles which reflected the light strongly.

5. *Eight days after feeding.*

The size has increased and segments are clearly seen, but there is no differentiation of the reproductive organs.

6. *Ten days after feeding.*

A gradual increase in size; the uteri being filled with what seemed to be primitive eggs, the structure of which was not very clear.

7. *Fourteen days after feeding.*

Growth is complete and the posterior gravid segments are so full of eggs that the testes are compressed and the excretory tubes obscure.

8. *Fifteen to nineteen days after feeding.*

Although there was some difference in the development of the dwarf tapeworms according to the host employed (mice, rats) they grew little by little, and about the seventeenth or eighteenth day, the experimental animals began to evacuate eggs in the faeces.

After eighteen days the last segments of the adult tapeworms become more slender, as the result of having discharged many eggs.

EXPERIMENTAL INFECTION IN MONKEYS

15 June, 1919. Two young male monkeys (one year and two months old), showing no tapeworms' eggs in their faeces, were obtained. I made one of the monkeys swallow many eggs of the dwarf tapeworm, but found no eggs on examining its faeces fifteen to sixteen days after the feeding ; so I again made it swallow many eggs. It was killed seven days later ; the contents and villi of its small intestine were closely examined, but no dwarf tapeworms were to be found.

26 July, 1919. The other monkey was fed with many dwarf tapeworms' eggs ; it became gradually feeble, and died in about six days. Examination of its small intestine showed thirteen young dwarf tapeworms in the lower part, the shape and size being no different from those of the young dwarf tapeworms in the small intestine of the mice and rats.

SWALLOWING OF EGGS BY MAN

29 January, 1919. Taking great care not to destroy the eggs of the dwarf tapeworm, I washed them with water, put many of them into capsules and swallowed them myself.

As a control, a part of the same material was given to a rat and a white rat which seven days later proved to be infected, but in spite of having examined my own faecal matter several times, for fourteen or fifteen days after ingestion, I could not find any dwarf

tapeworm eggs. Then I tried to expel tapeworms from my intestine, but unsuccessfully. Afterwards I swallowed eggs three times but neither eggs nor worms were found in my faeces.

Fortunately, a girl four years old was available for experiment; she was strongly-built, well nourished and healthy. I carefully examined her faeces several times, and each time found a few eggs of Ascaris but none of the dwarf tapeworm. On 12 April, 1919, she swallowed many eggs of the dwarf tapeworm in capsules and afterwards her faeces were examined on many occasions. On the first of May, nineteen days later, I found a few eggs of the dwarf tapeworm, and upon expelling the worms from her small intestine, I secured ninety-seven adult dwarf tapeworms.

SUMMARY

1. The six-hooked larvae of the dwarf tapeworm, about ten hours after ingestion, penetrate the villi in the upper part of the small intestine and four days later become encysted larvae (Cysticercus). Five days after ingestion, well developed larvae emerge from their cysts and leave the villi.

2. After seven days, well developed young dwarf tapeworms have some segments at the end of the body.

3. After nine days, their reproductive organs become visible.

4. About fourteen days after ingestion, the segments are full of eggs, in each of which can be seen a six-hooked larva (Onchosphere).

5. After about seventeen days, the ripe eggs of the dwarf tapeworms are found in the faeces of the experimental animals.

6. These experiments show that dwarf tapeworm eggs which have been evacuated with the faeces of the host, are swallowed by animals or man with their food, and that the eggs hatch and the six-hooked larvae are liberated in the small intestines, and later enter the villi of the small intestines where they become encysted larvae; then emerging from the cysts they grow into young dwarf tapeworms and upon maturity evacuate the ripe eggs.

7. Therefore, without any intermediate host, the dwarf tapeworm can directly develop in the body of mice, rats, young monkeys, or man, especially children.

A SPOROZOON OF *PHLEBOTOMUS PAPATASII*

BY

S. ADLER

AND

O. THEODOR

(*Microbiological Institute, Jerusalem*)

(*Received for publication* 9 *July*, 1925)

During a routine examination of 1,037 *Phlebotomus papatasii*, of which 939 were females, one female was found to contain oocysts. All the insects were collected in Jericho between April and June, 1925. The infected specimen was one of a number used for experimental purposes and dissected immediately after a feed on a laboratory assistant on 25th May, 1925.

On dissecting the head from the thorax a large number of small glistening bodies were seen to emerge from the thorax and these on examination proved to be sporocysts from a ruptured oocyst.

Further dissection revealed the fact that thorax and abdomen contained four ripe oocysts, one in the thorax and three in the abdomen. The oocysts were 130μ by 95μ in size and contained about a hundred sporocysts. Between the sporocysts were a number of round refractile bodies up to 8μ in diameter. The sporocysts varied in size from 21·4μ to 36·4μ in length by 15·7μ to 20μ in breadth and contained four to sixteen sporozoites and a residual body, from 3·6μ to 6·4μ in diameter, enclosed in a definite membrane. Apart from the residual body each sporocyst contained a number of small refractile granules lying apparently in the sporozoites.

In the sporocysts the sporozoites were seen to be actively motile, in some cases sufficiently so to cause the whole sporocyst to spin.

From the ruptured sporocysts sporozoites were seen emerging; each sporozoite was then observed to be lying in a membrane which when released from the sporocyst assumed the form of an elongated

spindle about 35μ in length and 6·4μ in breadth (figs. 6 to 8, and 12). The small refractile granules noted in the sporocyst were found to lie in the membrane outside the sporozoite, each membrane containing two to four granules.

The membranes, including the sporozoites, were all seen to be divided longitudinally by two fine lines (fig. 12). The sporozoites were actively motile within their membranes, constantly changing their shape and size by a series of contractile movements, so that it is difficult to give definite measurements. When fully stretched out the sporozoites were sickle-shaped with one end pointed and the other blunt, and then measured 34μ in length by 5μ in their thickest part (fig. 9). Each sporozoite contained a round nucleus.

By contracting and thus increasing their transverse diameter the sporozoites stretched the enclosing membrane and created a gap between the two longitudinal lines of the membrane (fig. 8). The sporozoites then slowly worked their way through the gap, leaving an empty husk containing several refractile granules (fig. 12).

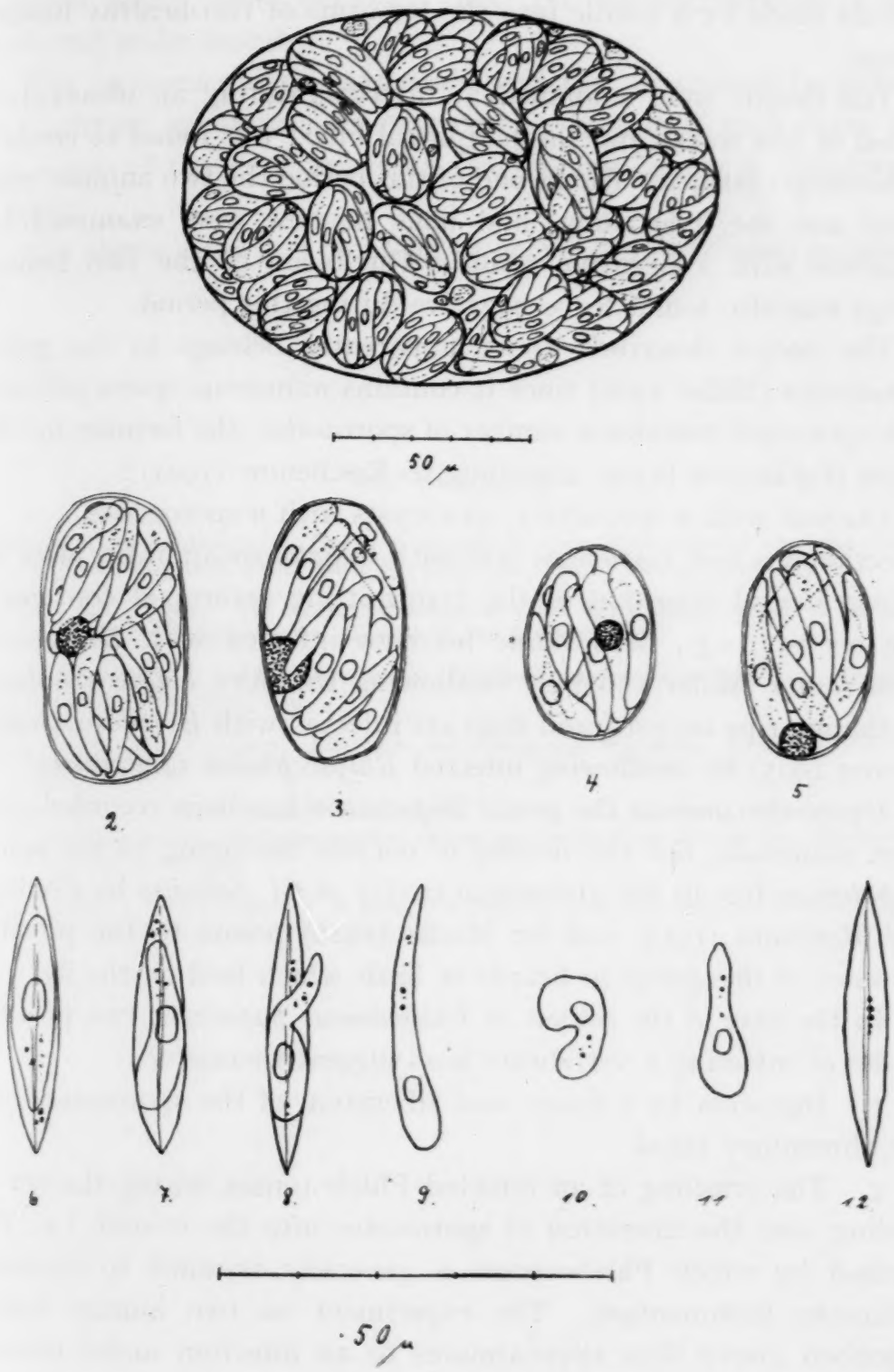
Having escaped, the sporozoites continue their contractile movements, constantly changing their shape and, at the same time, performing a slow translatory movement.

All the material was transferred to two slides and examined in the fresh.

As it seemed obvious that the oocyst above described formed a part of the life-cycle of a haemogregarine of a vertebrate, the following experiments were immediately performed.

(i) The contents of one fresh preparation containing intact sporocysts and numerous free sporozoites from ruptured sporocysts were carefully washed off the slide in 0·5 c.c. normal saline ; 0·25 c.c. of the resulting mixture was injected intraperitoneally into a specimen of *Gongylus ocellatus*, and the remainder intraperitoneally into a specimen of *Mabuia quinquefasciata*. The above two lizards were both free from haemogregarines at the time of the experiment. The gecko *Hemidactylus turcicus*, which is common in houses in Jericho and feeds on sandflies, would have been a more suitable animal for the experiment, but unfortunately no specimen of this animal was at the moment available in the laboratory.

(ii) The material from the second fresh preparation containing numerous sporocysts and free sporozoites was rubbed into puncture



FIGS. 1-12.

1. A complete oocyst.
2-5. Sporocysts.
6-7. Sporozoites in their enclosing membrane.
8. Sporozoite escaping from membrane.
9-11. Change in shape of a sporozoite.
12. Empty membrane with refractile granules.

wounds made by a needle into the forearms of two healthy human beings.

The lizards were examined at intervals during an observation period of five weeks and the peripheral blood was found to contain no haemogregarines. At the end of this period the two animals were killed and the liver, lungs and bone marrow were examined for schizonts with a negative result. The blood of the two human beings was also found to be negative during this period.

The oocyst described above apparently belongs to the genus *Hepatozoon* (Miller 1908) since it contains numerous sporocysts and each sporocyst contains a number of sporozoites, the formula for the genus *Hepatozoon* being, according to Reichenow (1921) :

' Oocysts with *n* sporocysts, sporocysts with *n* sporozoites.'

Infection of a new vertebrate host with *Hepatozoon* sp. takes place by the accidental ingestion of the transmitting arthropod containing ripe oocysts, e.g., *Mus rattus* becomes infected with *Hepatozoon perniciosum* (Miller 1908) by swallowing the mite *Lelaps echidnius* containing ripe oocysts, and dogs are infected with *Hepatozoon canis* (James 1905) by swallowing infected *Rhipicephalus sanguineus*.

Up to the present the genus *Hepatozoon* has been recorded only from mammals, but the finding of oocysts belonging to the genus *Hepatozoon* free in the abdominal cavity of *Gl. palpalis* by Chatton and Roubaud (1913) and by Macfie (1916) points to the possible presence of this genus in lizards or birds which feed on the fly.

In the case of the oocyst of *Phlebotomus papatasii*, two possible modes of infecting a vertebrate host suggest themselves :

1. Ingestion by a lizard and liberation of the sporozoites into the alimentary canal.

2. The crushing of an infected Phlebotomus during the act of feeding, and the liberation of sporozoites into the wound, i.e., the method by which Phlebotomus is generally assumed to transmit cutaneous leishmaniasis. The experiment on two human beings described above thus approximates to an infection under natural conditions.

A number of observers (Krempf 1917, Dimond 1917, Sergent, Et. and Ed. and Parrot 1922, Noc 1922, Nattan-Larrier 1922), have described haemogregarines from man. The findings of all these authors have been subjected to a destructive criticism by

Wenyon (1923), who concluded that ' the haemogregarines of man have still to be found.'

The bionomics of *P. papatasii* render it an eminently suitable transmitting agent of a haemogregarine to man if such occur and the result of the experiment on two human beings is therefore of interest.

The above is the first record of an oocyst in *Phlebotomus papatasii*.

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THE GENUS *TETRACAMPOS* WEDL, 1861

BY

T. SOUTHWELL

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Woodland, in the '*Annals of Tropical Medicine and Parasitology*,' Vol. XIX, No. 2, p. 185, refers the above genus to the *Bothriocephalidae*.

In a former issue of the 'Annals,' I gave certain reasons for referring the genus to the Order *Cyclophyllidea*.

This difference of opinion cannot, unfortunately, be settled by an examination of the worm in question, viz., *T. ciliotheca* Wedl, 1861, because the material is not available.

As the species is stated by Wedl to possess four lappets or bothridia which are figured, it should, on that account, be referred to that Order of Cestodes which is characterised by the possession of four bothridia, viz., the *Tetraphyllidea*. Owing to the fact that Wedl's figure of the head leaves one in considerable doubt as to whether the so-called bothridia are really bothridia, or whether, on the other hand, they are badly figured acetabula; and also having in mind the fact that other cestode parasites with armed heads bearing true acetabula, and with ventral pores, have been repeatedly obtained from fish closely related to that in which *T. ciliotheca* was found, the writer concluded that Wedl's genus *Tetracampos* belonged to the *Proteocephalidae;* and, as in this family the head is armed with four suckers, it was referred to the Order *Cyclophyllidea*.

Up to the present helminthologists have agreed, and rightly, that the primary divisions of the polyzootic cestodes should be made on the character of the head. Thus, in the *Cyclophyllidea* the head bears four suckers, in the *Tetraphyllidea* four bothridia or lappets, in the *Trypanorhyncha* four proboscides, and in the *Pseudophyllidea* sometimes one or more, but usually two, bothria (or grooves).

The head thus provides a ready and eminently satisfactory means of effecting a natural classification of this group of worms into

Orders, and the utility and simplicity of this means of classification justifies us in retaining it, until a better system is provided.

In the absence of a head, it is frequently impossible to refer a cestode worm to the Order to which it belongs. If the genital pores (excluding the uterine pore or pores, whether primary or secondary) are situated on the ventral surface, the worm is placed in the Order *Pseudophyllidea ;* there are, however, exceptions to this rule.

If the genital pores are lateral, then it is necessary to locate the position of the vitelline glands. If this organ consists of numerous follicles situated laterally, it is still impossible to say whether the worm belongs to the Order *Tetraphyllidea* or to the Order *Trypanorhyncha.*

If the gland is single, the worm is referred to the Order *Cyclophyllidea.* Unfortunately, however, there are a number of species which, although they possess a head typical of the *Cyclophyllidea,* have the vitelline glands arranged along the lateral margins, and there are also a few species which, while characterised by having a Tetraphyllidean head, have the vitelline glands condensed into a single mass situated behind the ovary.

The male and female genital organs are of the same type, especially in species of all the three Orders, *Cyclophyllidea, Tetraphyllidea* and *Trypanorhyncha,* the trivial differences which exist being limited to the disposition of the musculature, the number of testes, the size of the cirrus pouch, the position of the pore on the lateral margin, etc. —points obviously only of importance in the differentiation of species, or at most of genera. The form of the uterus in the *Pseudophyllidea* is, however, usually characteristic in that Order.

In spite of the fact that in *T. ciliotheca* the head bears four bothridia, or four suckers, Woodland refers the genus to the Order *Pseudophyllidea,* and states that ' scolex characters count for very little.'

Woodland realises that the head of a Bothriocephalid usually possesses two bothria, for he states that the four bothridia in *T. ciliotheca* ' are evidently the four walls bordering the bothriae or sucking grooves.' For a similar reason one could consider the Order *Tetraphyllidea* identical with the *Pseudophyllidea.*

It is true that Wedl states that in *T. ciliotheca* the embryophore is ciliated exactly as it is in *D. latus.* Practically nothing is known

regarding the eggs of the *Tetraphyllidea*, and for this reason one cannot say whether the fact that the embryophore in *T. ciliotheca* is ciliated, has any particular significance or not.

Woodland states that other typically Bothriocephalid features of *T. ciliotheca* are: (1) the shape of the anterior proglottides; it is not stated what this character is, and the writer's experience is that the anterior segments are almost always featureless; and (2) the ventral position of the genital apertures. It has already been pointed out that the uterus in many species of *Proteocephalidae* bursts to the exterior by a slit or a number of slits on the ventral surface, and it is not impossible that what Wedl called a genital pore was a uterine opening.

Referring to the *Proteocephalidae*, Woodland further writes 'for me the possession of lateral vitelline strands and of ventral uterine pores affords two very good reasons for relegating the family to the *Tetraphyllidea*.' It is common knowledge amongst all who have worked with worms of this order, that although in gravid segments the uterus sometimes bursts to the exterior by a slit or slits situated on the ventral surface, the presence of true uterine pores has only been established in about six species. Further, the vitelline glands are not in every case situated laterally.

Woodland's paper is useful in that his figures help one to realise pointedly the wide difference between the head of *T. ciliotheca* and those of the two other species which he considers so closely allied to it.

A NEW MEDIUM FOR THE DIFFERENTIATION OF *B. COLI* IN WATER ANALYSIS

BY

J. L. PAWAN

GOVERNMENT BACTERIOLOGIST, TRINIDAD, B.W.I.

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During the course of an investigation into the bacteriology of the water supplies of the colony the repeated isolation of *B. coli* (lactose +, indol +) and of Houston's atypical *B. coli* from small quantities (1 c.c., ·1 c.c., and ·01 c.c.) of raw natural waters which were declared on sanitary survey to be free from all possibility of human faecal pollution and very remotely, if at all, liable to any animal contamination, centred attention on the sufficiency of the methods for the bacterioscopic examination of waters recommended by a Committee of the Royal Institute of Public Health in 1903, and adopted by bacteriologists in England and elsewhere as the standard method, and the interpretation of the results obtained by this method.

The literature of the bacteriology of tropical waters reveals frequent references to the presence of *B. coli* as detected by the standard method in waters which would be certified as pure and free from objectionable pollution by the comparative freedom from water-borne diseases among those drinking such waters, and by the results of sanitary surveys.

Daniels (1908), working on the waters from jungle streams of the Federated Malay States, observed that it was exceptional to find a jungle stream from which *B. coli* could not be isolated in 2 c.c., even though such waters would appear to be free from human and animal faecal pollution. Archibald (1910), commenting on the waters of the Soudan, remarked :

> ' If samples of water taken from shallow wells or rivers in the Tropics are subjected to a few simple tests for the presence of faecal contamination, the results will often show such a state of things that no analyst in England would ever consider the passing of such waters as fit for human consumption, and yet the water from those sources is used daily by both Europeans and natives alike without any ill effects to health as far as can be told. The question naturally arises whether in the face of these existing conditions one would be justified in using European standards of water purity as a guide or whether some modification of the European standard could be generally employed in tropical climes ? '

Wise and Minett (1912) isolated *B. coli* in from 1 to ·001 c.c. of raw waters from various sources used for drinking purposes by the inhabitants of British Guiana. Clemesha in India (1908-1912), struck by the high degree of 'faecal' (?) pollution in the drinking water supplies of the Madras Presidency as evidenced by the presence of *B. coli* isolated by the standard method, conducted a series of interesting experiments demonstrating the value of sunlight in the process of purification of the waters of India.

B. coli as defined by the standard method, and by Houston, embraces more than ten varieties of organisms, and though one or more varieties may be present in a water supply, the natural purification which that water undergoes from exposure to sunlight destroys those organisms which are objectionable or are evidence of objectionable pollution.

Hence the mere statement by a water analyst of the isolation of *B. coli* in a certain quantity of water is, as regards the Tropics, at all events, of comparatively little value from the sanitary point of view unless the effects of self-purification are taken into account. If, he says, the standard method be applied in its entirety to India, nearly every drop of drinking water in that country would be condemned.

In England the raw waters of the Thames investigated by Houston are waters to which sewage and other pollution gain constant access and *B. coli* in such waters would almost invariably be of faecal origin. Unfortunately the literature available locally does not record the result of quantitative examinations for the presence of *B. coli* in the raw natural waters of England, waters obtained from uninhabited regions and not exposed to human or animal faecal pollution.

Thresh, however, refers to a public water supply from the Welsh moorlands *in which no sewage contamination* was possible, but in which *B. coli* (lactose +, indol +) was present in ·1 c.c. Such a water, he recommends, should be filtered before being delivered to the consumer. Does this example, though solitary, indicate that *B. coli* may perhaps be present in raw natural waters not exposed to human or animal pollution in temperate regions as is the case in the Tropics?

The standard method postulates that all *B. coli* are of faecal

origin without regard to the fact that such organisms are frequently found not only in small quantities of raw natural waters free from all faecal pollution, but also in *unpolluted soil*. From the soil free from faecal contamination these organisms may gain entrance to water supplies, and such supplies may be condemned by the standard method as polluted and unfit for human consumption. Chen and Rettger (1919) found 156 out of 467 (33·4 per cent.) coli-like organisms isolated from unpolluted soil to be lactose +, indol + organisms and Max Levine 37·3 per cent. out of 177 lactose fermenters of the soil to be also indol producers.

In Trinidad, of 120 cultures isolated from unpolluted soil by the standard method 42 per cent. were typical *B. coli* (lactose +, indol +) and if Houston's atypical *B. coli* be included, the percentage of *B. coli* regarded as an index of faecal pollution would be considerably higher.

If lactose +, indol + *B. coli* may be isolated by the standard method from soils to which no faecal matter has gained entrance, is a method which does not attempt to differentiate between faecal and non-faecal *B. coli* sufficient to justify an analyst in expressing an opinion upon the sanitary quality of a water, particularly if the sanitary control of that water is liable to variation ? At all events, should the water analyst not attempt to indicate the faecal or non-faecal origin of *B. coli* isolated from water ?

Keyes, Rogers, Clarke and others (1909-1914) showed by accurate determination of the gas volumes and gas ratios produced in the anaerobic fermentations of glucose that the non-spore-bearing lactose fermenters of faeces can be divided into two groups, one a low ratio or *B. coli* group in which the proportion of CO_2 to H_2 is almost constantly equal to 1·06 and the other a high ratio or B. aerogenes-cloacae group which produces considerably more CO_2 than H_2, with a wide range of ratio between these gases.

Clark and Lubs (1915) showed that in a carefully adjusted sugar medium the low ratio organisms produce a relatively high hydrogen ion concentration which can be recognised by an indicator, such as methyl red becoming red, whilst the high ratio organisms produce a low hydrogen ion concentration and methyl red becomes yellow.

In human faeces, according to Rogers, Clarke and Lubs, the low ratio group (methyl red +, Voges-Proskauer −) constitutes

74 per cent., and the high ratio group 26 per cent., of the lactose fermenters, whilst in bovine faeces the low ratio group constitutes 99·4 per cent., and the high ratio group ·6 per cent. (Rogers).

Chen and Rettger, in 1919, found all of 173 organisms from faeces to be methyl red positive and Voges-Proskauer negative.

In Trinidad, of 740 cultures isolated from human, bovine, and equine faeces, 94 per cent. were methyl red positive and all Voges-Proskauer negative.

On the other hand, Chen and Rettger found that of 467 coli-like organisms isolated from unpolluted soils, 430 belonged to the high ratio (methyl red —, V.-P. +) or B. aerogenes-cloacae group, and 20 to the low ratio *B. coli* group and, as stated above, 33·4 per cent. of these 467 were lactose +, indol +.

In Trinidad, of 120 cultures isolated from unpolluted soil, 85 per cent. were methyl red negative and 15 per cent. methyl red positive, and as previously pointed out 42 per cent. were lactose +, indol +.

But the gas ratio determination is not possible in the ordinary laboratory analysis of water, and whilst the methyl red and Voges-Proskauer tests have been found in the case of faeces and soil to indicate fairly accurately the habitat of the organism under investigation, their application in the bacteriological analysis of waters has been shown to afford no clue as to the source (faeces or soil) of the organism isolated from a water. Thus Winslow and Cohen found the percentage of methyl red positive, Voges-Proskauer negative organisms to be practically the same in polluted, unpolluted and stored raw waters. Out of 255 coli-like organisms, 76 per cent. from unpolluted, 77 per cent. from polluted and 85 per cent. from stored rain water were methyl red positive and V.-P. negative. Stewart Koser found 80·4 per cent. of the colon group cultures obtained from polluted waters and 73·3 per cent. from unpolluted waters to be methyl red positive and Voges-Proskauer negative.

In Trinidad, of 220 organisms isolated from polluted waters, 87·3 per cent. were methyl red positive, 6·3 per cent. methyl red negative, and 6·4 per cent. doubtful; and of 240 cultures obtained from sanitarily pure waters 42·5 per cent. were methyl red positive and 57·5 per cent. negative. Though the American Public Health Association (1923 ed.) recommends the methyl red and Voges-Proskauer tests in the bacteriological examination of water, local

experience supports the conclusion of Winslow, Cohen, Stewart Koser and others that the lack of correlation between these tests and the sanitary qualities of waters justifies little reliance being placed upon them as indices of sanitary purity.

Stewart Koser (1923), in a study of the utilisation of salts of various organic acids, found that the two sections of the colon group of organisms could be clearly distinguished by the use of a chemically definite medium containing sodium, potassium or ammonium citrate as the only source of carbon. Such a synthetic medium can be made by dissolving 1·5 grammes microcosmic salt Na (NH_4) PO_4 + H_2O, 1 gramme KH_2 PO_4, 0·2 gramme $MgSO_4$ and 2 grammes sodium citrate in 1000 c.c. distilled water, tubing, and autoclaving at 120° C. for fifteen minutes. A clear colourless liquid is obtained. In this medium Stewart Koser found that 90·7 per cent. of *B. coli* isolated from faeces failed to develop, whilst the B. aerogenes-cloacae group produced a visible turbidity within forty-eight hours at 30° C. This differentiation correlated with the methyl red and Voges-Proskauer tests as far as the typical *B. coli* type and the aerogenes section in faeces are concerned.

With regard, however, to organisms isolated from the soil, he found that a number were consistently methyl red positive and Voges-Proskauer negative, although they had been obtained from soils regarded as free from pollution. When tested in the citrate medium these soil coli were found to utilise it. Of 72 cultures obtained from unpolluted soils, 97·2 per cent. utilised the citrate with the production of a visible turbidity and were distinct from faecal *B. coli*, whilst the methyl red test showed 51·4 per cent. alkaline to methyl red and the Voges-Proskauer 52·8 per cent. positive.

In Trinidad, of 432 cultures isolated from human, bovine, and equine faeces, 96·3 per cent. failed to develop in the citrate medium, while 3·7 per cent. did so; and in the case of unpolluted soils, of 214 cultures of the coli group, 90 per cent. utilised the citrate medium in forty-eight hours and 10 per cent. failed to do so. The citrate medium as a biological test is thus an accurate indicator of the habitat of the coli organism isolated from faeces and soil. In the application of the citrate medium for the differentiation of faecal and non-faecal *B. coli* obtained from waters, very striking results have been obtained. Samples of waters were secured from localities

in Trinidad where the chances of human intestinal pollution were impossible and contamination by birds and an occasional wild animal practically negligible ; by the standard method, *B. coli* were isolated and put through the citrate, methyl red and Voges-Proskauer tests. Of 240 cultures thus obtained, 81·3 per cent. grew in the citrate medium and 18·7 per cent. failed to do so; whilst of 210 *B. coli* isolated at various periods from polluted streams below villages 90·9 per cent. failed to utilise the citrate and 9·1 per cent. produced a distinct turbidity. The citrate utilisation by *B. coli* is thus seen to afford some degree of correlation with the sanitary survey of a water supply. Further, the *B. coli* colonies (lactose +, indol +) isolated from a certain quantity of water, say 1 c.c., by the standard method, may, by the citrate test, be shown to be of non-faecal origin and it is only in a larger quantity of water (5·10 or 25 c.c.) that faecal (citrate —) *B. coli* (lactose +, indol +) is found. Whilst, therefore, by the routine standard method a water may be condemned, by the use of the citrate test the bacteriological analysis of a water supply may be found to harmonise with epidemiological and sanitary conditions. To those, therefore, engaged in water analysis in the Tropics, particularly of those waters where that perfect sanitary control obtained by Sir Alexander Houston for the waters of the Metropolitan Water Board can only be an impossible vision, Stewart Koser's remarks should be of special interest. He says :

' the primary results shewn by the citrate medium indicate that this method of differentiation is deserving of further study with regard to its usefulness and application in the sanitary examination of water supplies, though the final acceptance of any such test must of course await general confirmation at the hands of different workers.'

Such a test is necessary. For is the relatively low incidence, in certain parts of the Tropics, of water-borne diseases, in contrast with the high degree of faecal pollution as evidenced by the presence of *B. coli*, detected by the standard method, due to the constant accidental absence of the specific pathogenic organisms or to the natural purification which waters in the Tropics undergo from exposure to sunlight in addition to the fact that by the standard method no attempt is made to differentiate between faecal and non-faecal *B. coli* ?

SUMMARY

1. *B. coli* (lactose +, indol +) may be isolated by the standard method not only from faeces and polluted waters, but also from unpolluted soils and unpolluted waters.

2. As a standard indicator of faecal contamination its value is not therefore unquestionable.

3. Local experience indicates that the utilisation of citrate by *B. coli* may be of value in differentiating faecal from non-faecal *B. coli* in water analysis.

UNPOLLUTED SOIL

	In Trinidad		In America (Chen and Rettger)		In America (Levine)	
		No. of Colonies studied		No. of Colonies studied		No. of Colonies studied
Lactose + Indol + *B. coli*	42 per cent.	214	33·4 per cent.	467	37·3 per cent.	177

CITRATE TEST IN AMERICA (STEWART KOSER)

	Growth in Citrate	No growth in Citrate	No. of Colonies studied
Faeces...	9·3 per cent.	90·7 per cent.	118
Unpolluted Soil ...	97·2 per cent.	2·8 per cent.	72

CITRATE TEST IN TRINIDAD

	Growth in Citrate	No growth in Citrate	No. of Colonies studied
Faeces...	3·7 per cent	96·3 per cent.	432
Polluted Water ...	9·1 per cent.	90·9 per cent.	210
Unpolluted Soil ...	90 per cent.	10 per cent.	214
Unpolluted Water ...	81·3 per cent.	18·7 per cent.	240

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MALARIA INFECTION AS IT OCCURS IN LATE PREGNANCY; ITS RELATIONSHIP TO LABOUR AND EARLY INFANCY

BY

D. B. BLACKLOCK

AND

R. M. GORDON

(*From the Sir Alfred Lewis Jones Research Laboratory, Freetown, Sierra Leone*)

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PLATE VI

I. INTRODUCTION

In a previous paper (1925) we published an account of the malaria incidence in a series of twenty-six placentas of native women in Freetown. The investigation of placental malaria has been continued on all material available since then, so as to eliminate seasonable variations, and the records now cover a period of a complete year, i.e., from July, 1924, to July, 1925. Examinations have been made not only of films of the placental blood and of the peripheral blood of the mother at and about the time of labour, but also of cord and peripheral blood films of the children born of mothers with infected placentas. A small amount of material has been obtained from post mortem examination of children born dead or who died within a period of seven days. For purposes of comparison certain figures of the infection rate of the adult male population have been introduced.

The distribution of parasites in infected placentas has been studied with a view to discovering whether the whole placenta is equally infected or whether there is special concentration of the parasites in any particular areas.

Evidence of the transmission of parasites from the placenta to the child has again been sought. Although evidence of such transmission of parasites has never once been obtained throughout the whole series, yet there are certain facts which strongly suggest that the presence of malaria in the placenta is frequently associated with abnormal labour, that the death-rate among children born of mothers with infected placentas is unusually high, and that the

blood of the child is deleteriously affected by the parasitic invasion of the placenta. Little material from cases of abortion was available and what was obtained was not usually suitable for examination. It is not possible, therefore, to adduce any facts to show whether malaria is an important factor in the causation of abortion in Freetown or not. This is an aspect of the case which clearly requires attention, but until greater facilities for obtaining material are made it is not possible to advance much in this direction.

In our previous paper, we discussed the view held by some observers that new-born children of infected mothers possess a temporary and partial tolerance as regards malaria, so that a new-born child, although congenitally infected, does not present parasites in the peripheral blood at birth nor until after the lapse of a certain time. We argued that there was no direct evidence of the existence of such a partial tolerance on the part of the child, and that there was evidence against its existence at least in some cases, i.e., where authentic congenital malaria has been demonstrated. We noted, however, that of 41 children of one month or under, only one, a child between three and four weeks old, had parasites in the peripheral blood. In this present series it will again be seen that children under one month rarely show parasites in the peripheral circulation. This freedom from parasites in the peripheral blood may be due to freedom of the child from infection. If this is so, it may merely result from the fact that, for some unexplained reason, children up to a week or two old are little exposed to the bites of infected anophelines. On the other hand, it would be compatible with either a temporary general immunity, i.e., a condition during the existence of which the child is totally incapable of developing infection anywhere, or a condition of local immunity with partial tolerance, i.e., a condition in which the child is in fact infected, but in which the infection does not appear equally distributed throughout the body, but only in certain parts, of which the peripheral circulation is not one. That such local infections do occur in adult women, and, moreover, that they can very frequently exist without the production of obvious constitutional symptoms, we are able to prove conclusively from the present series.

We shall show that of 150 parturient native women, aged 15 to 42, examined during the period of twelve months, 55 proved to be

infected with *P. falciparum*. Of those infected, however, only 10 showed infection of both peripheral and placenta blood. In the remaining 45 only the placenta was infected.

It must be concluded, therefore, either that in these latter cases the parasites remain localised in the placenta, and never leave it, or else that if they do leave the placenta on their way to the peripheral blood via the vena cava, they are rapidly destroyed ; for it seems impossible to explain merely on the ground of dilution, the non-appearance of parasites in films from the peripheral circulation, when we consider that the placenta is a highly vascular organ, that it represents some $\frac{1}{120}$th of the total body weight and that of the maternal erythrocytes which it contains as many as 65 per cent. may be infected, as was shown by us previously (1925). See Plate VI, fig. 1.

It seems not only legitimate but necessary to believe that in pregnant native women infected with malaria, there are certain portions of the circulatory system which are immune from infection ; while at the same time, in the same individuals, other portions, far from being immune, exhibit massive infection, accompanied by active sporulation. If we admit a local immunity in the case of the mother, it must be admitted that a similar condition may exist in the child. Although in no case in a child born of a mother with placenta infected were parasites found either in the cord, peripheral blood, or in such organ smears as were available, we are not in a position to deny the possibility of malaria parasites establishing themselves in the internal organs of the child although not appearing in its peripheral blood. We can say, however, that such a condition, while it would be in accordance with the idea of the existence of local immunity in one portion of the child's circulation, namely, the peripheral blood, would equally imply the absence of such an immunity in another portion, namely, the umbilical cord. This question will be referred to again in discussing the age incidence of malaria infection in the children, and the fact that in a few cases of children born dead or who died immediately after birth, there was found in smears made from the internal organs, pigment which could not be distinguished from malaria pigment.

Before proceeding to a detailed account of the facts obtained, it may be noted as somewhat extraordinary that since the observa-

tions made on placental infection by the Greek observers Pezopoulos and Cardamatis (1907), little attention has been given to the discrepancy which appears to exist between the infection rate of males and females as judged by the peripheral blood rate of the former and the placental blood rate of the latter; nor, in our opinion, has sufficient attention been attached to this method of diagnosing malaria in the case of parturient women whose peripheral blood has yielded no evidence of it.

II. EXAMINATION OF THE PERIPHERAL BLOOD

A. Of mothers.

Thin film preparations of blood were made from the peripheral blood of 173 mothers at the time of labour; in addition to thin films, thick films were also examined in 71 of these cases. The number of cases in which malarial infection was diagnosed by examination of the peripheral blood was 12, of which 9 had parasites of *P. falciparum*, and 3 pigmented leucocytes only; it is noteworthy that in no case were gametes found, although in 5 of the 9 positive parasitic cases, one or more thick films were also examined.

Seasonal incidence of infection in the peripheral blood of mothers.

The number of mothers examined by films of the peripheral blood varied from 5 to 24 in a month; the total number examined and the approximate percentage found positive in each month are shown in Table I.

TABLE I.

Showing monthly total of mothers examined and percentage positive.

	Total	Percentage positive		Total	Percentage positive
July	8	25	January	17	6
August	5	20	February ...	5	0
September ...	10	10	March	7	0
October	16	0	April	27	7
November... ...	24	8	May	25	4
December ...	13	8	June	16	13

B. Of non-parturient women.

Of 43 women of the age of 16 and upwards, all, however, examined by means of thick film preparations from the peripheral blood, three had parasites. One of these was a *Plasmodium vivax* infection, the other two were *P. falciparum* infections, and in one of the latter crescents were present.

C. Of Adult males.

In a series of 150 males of the age of 16 and upwards, all examined by the thick film method, three had trophozoites of *P. falciparum* in the peripheral blood; in no case were crescents found.

D. Of children.

Each one of a series of 809 children of all ages up to two years and a half was examined, on its first appearance, by thin film preparations of the peripheral blood; an additional examination was made at the same time in the case of 100 of these children by the thick film method. Of the 809 children, 169, i.e., 20.9 per cent. had parasites in the peripheral blood; *P. falciparum* occurred alone in 149 cases, *P. malariae* alone in 12 cases, and *P. vivax* alone in 2 cases; mixed infection of *P. falciparum* and *P. vivax* occurred in 2 cases, of *P. falciparum* and *P. malariae* in 2 cases, and of *P. falciparum*, *P. malariae*, and *P. vivax* in 1 case. One case diagnosed by pigment alone cannot be classified. *P. falciparum* infection was found therefore in 19.0 per cent. of the 809 cases examined. Crescents were present in 23 cases, i.e., 14.9 per cent. of the 154 *P. falciparum* cases; this percentage is low, as in cases in which trophozoites were found at once, the examination was not continued to the time limit prearranged.

Seasonal incidence of malaria in the peripheral blood of 809 *children up to* $2\frac{1}{2}$ *years.*

The monthly total number of new children examined by films of the peripheral blood varied from 36 to 104; the total examined and the percentage found positive in each month are shown in Table II.

TABLE II.

Showing monthly total of new cases examined and percentage positive.

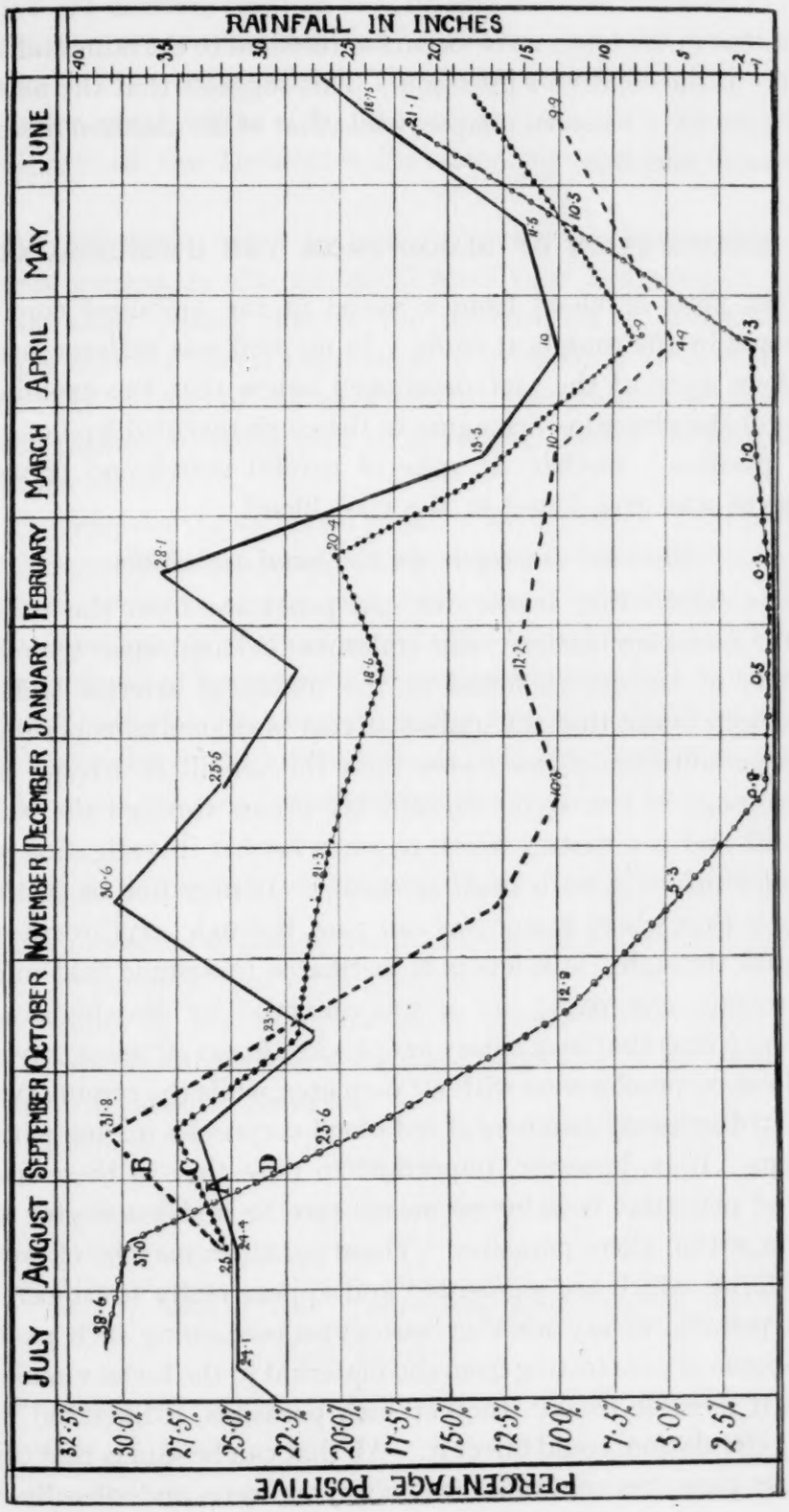
Month ...	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May	June
Total ...	62	78	93	60	36	51	77	57	104	40	87	64
Per Cent. positive...	24·1	24·4	26·8	21·7	30·6	25·5	22·1	28·1	13·5	10·0	17·2	18·7

Each case on its first appearance is classified here as new; on any subsequent appearance, therefore, it is classified as old, and the parasitic findings require separate record, as is shewn in the graph given below.

The number found positive among new cases in each month is expressed in Graph I A as a percentage of the total new cases appearing for examination for that month. The number of old cases found positive for the first time in each month is expressed in Graph I B as a percentage of the corrected total of old cases seen in that month. The corrected total of old cases is arrived at by excluding all cases which had previously been found positive. The numbers found positive in the above two categories are added together and expressed in Graph I C as a percentage of the total cases, i.e., new cases plus corrected old cases, appearing for examination in each month. The rainfall in inches in an average year is shown in Graph I D.

Table II and Graph I A, which give the same information, represent the results of a single examination; Graph I B represents the results of at least two, and it may be numerous examinations and includes all cases which, negative on first examination, proved positive at a later time; the resultant curve of the summation of the positives in A and B shown in Graph I C gives a more accurate impression of the seasonal incidence of malaria in the children than does A alone or B alone.

It is seen that the form of curve C which we regard as yielding the most reliable information on the question of seasonal incidence in children, presents a fairly definite relationship to the rainfall curve. The relationship is such as to show that soon after the commencement of the rains the malaria incidence rises. If we compare the peripheral



GRAPH I.

Showing seasonal incidence of Malaria and average rainfall in inches.

blood of children with the placentas of mothers (Section IV, Table V) we see that in the latter a rise occurs antecedent to the rains and before the rise in the children's infection. This suggests that the placental rise represents a seasonal relapse, while that of the children represents a seasonal infection.

III. EXAMINATION OF BLOOD FROM THE UMBILICAL CORD

Thin films of blood from a vessel in the umbilical cord were examined in 162 umbilical cords. In no cord was malaria infection found, in spite of the fact mentioned below that the examination of 155 of the placentas belonging to the cords revealed 59, i.e., 38 per cent. positive; further, in spite of careful search, no pigmented leucocyte was ever found in the cord blood.

Maternal leucocytes in the foetal circulation.

It is stated that leucocytes can penetrate from the maternal into the foetal circulation; this statement is based upon the relative numbers of leucocytes found in the umbilical arteries and vein. It has been found that the umbilical vein blood contains per volume a greater number of leucocytes than the umbilical arterial blood. This passage of leucocytes presumably occurs through the walls of the villi and is a matter which requires further investigation where placental infection with malaria occurs. It may not be justifiable to argue that where leucocytes can pass through, erythrocytes can also pass through; still less is it justifiable to assume that infected erythrocytes can pass; for it was observed by Marchiafava and Bignami (1894) that in capillary apoplexies almost all the extravasated red blood-corpuscles were without parasites, while the cerebral vessels contained immense numbers of red blood-corpuscles having parasites in them. It is, however, important to note that in these heavily infected placentas it is by no means rare to find leucocytes which contain within them parasites. These parasites may be of any size up to forms which are segmented and appear ready to rupture. It is not possible to say whether leucocytes containing such parasites are capable of penetrating from the maternal to the foetal circulation, nor is it possible to say whether such parasites, if liberated in the child's circulation, could infect it. All that can be said is that certain of these parasites contained in leucocytes were undoubtedly alive at the time of examination as evidenced by their movements visible

by the dark ground illumination method. These parasites containing leucocytes are, comparatively speaking, rare, and it is unlikely that even if they penetrated into the child's circulation, they would come under observation. In a number of placentas examined the majority of the leucocytes contained pigment (see Plate VI, fig. 2).

If the difference noted by some observers between the total leucocytes present in the umbilical cord vein and arteries is, as stated by Gray (1923), due to the penetration of the maternal leucocytes into the foetal circulation, it is difficult to account for the fact that in spite of the most careful examination we failed to find such pigmented leucocytes in either the cord or the peripheral blood of the child.

In the endeavour to ascertain whether the condition of the arterial and venous cord blood, as regards the proportion of leucocytes present, is as stated, we have in one case made careful enumeration of the leucocytes present in films of each. The result showed that such difference as existed between the leucocyte content of the venous and the arterial cord blood was negligible, but showed a slight preponderance of leucocytes in the arterial blood, in the proportion of arterial 187 and venous 170 to 50,000 erythrocytes.

IV. EXAMINATION OF BLOOD FROM THE PLACENTA

Of 155 placentas of native women examined for malaria 59 were found to be positive, that is 38.0 per cent.

The results of the different blood examinations obtained so far are set out in tabular form arranged according to the ascending percentage of parasitic positives.

TABLE III.

Showing the parasitic findings of various groups.

	Blood films of	162 Umbilical cords	150 Adult males (peripheral blood)	43 Non-parturient women (peripheral blood)	173 Mothers (peripheral blood)	809 Children (peripheral blood)	150 Mothers' placental blood	155 Placentas
Percentage having	Parasites ...	0·0	2·0	7	6·9	20·9	36·6	38·0
	Crescents ...	0·0	0·0	2·3	0·0	2·8	0·0	0·0

It is interesting to compare the above placental figures with those given in Table IV taken from Clark (1915), which shows the distribution of placental infection among different races in his series of 400 labours.

TABLE IV.

400 Routine cases of labour.

Race of the women examined	No. examined of each race	No. of positive identifications of malaria	Per cent. of positive cases
North Americans (white)	118	0	0·0
Latin Americans (mestizo) ...	92	3	3·26 +
Europeans (white)	17	1	5·88 +
West Indian Negroes	173	15	8·67 +
Total	400	19	4·75

It is seen that Clark's percentage of positive mothers is low as compared with ours, 4·75 per cent. as compared with 36·6 per cent. A partial explanation of this fact is obtainable from consideration of the groups forming his total. Thus the 118 North Americans (white) give a percentage infected figure of 0·0, whereas the 173 West Indian negroes give the highest figure a percentage infected of 8.67. The difference of incidence is attributed by Clark to the higher hygienic plane of the North Americans, to the greater exposure to infection of the West Indian Negroes on account of their residential surroundings and their much lower hygienic and economic standard. Even taking the figure for West Indian negresses alone, however, the infection rate, i.e., 8·67 per cent. does not approach that seen in West African native women in Freetown, i.e., 36.6 per cent. If placental findings are taken as a criterion of malarial infection, it appears that the West African native women in Freetown are more than four times as frequently infected as the West Indian negresses dealt with by Clark.

Seasonal incidence of infection of the placenta.

The number of mothers whose placenta was examined by blood films varied from 6 to 24 monthly during the year. The total

number examined and the percentage found positive in each month are shown in Table V.

TABLE V.

Showing monthly total of mothers examined and the percentage found positive.

	Total	Percentage positive		Total	Percentage positive
July	8	62	January	18	39
August	6	33	February	7	43
September	12	42	March	11	18
October	16	19	April	11	18
November	24	38	May	15	53
December	13	31	June	9	56

Type of infection in the placental blood.

Without exception all the cases found positive in the placenta were infected with *P. falciparum ;* in one case a few parasites which resembled quartan were also found. In spite of prolonged examination of the placental films, no crescents were ever found in the placental blood. In writing the account of our first series of twenty-six placentas we drew attention to the absence of crescents in the placental blood, and also in the peripheral blood of such cases as showed infection there. We have obtained no evidence from this larger series that crescents are being formed elsewhere in these infected individuals, as no crescents have been found in any of them in the peripheral blood during, or immediately after, labour. If post-mortem material had been available it might have been possible to determine whether any crescents were present in internal organs. Blacklock (1921) produced evidence from a case of indigenous infection with *P. falciparum* in England that the bone marrow was the most suitable site for the development of crescents, a site which had previously been stated to be favourable by Marchiafava and Bignami and various other observers.

These cases, then, although the infection in the placenta is often very intense, are not producing crescents in this site. Nor does it appear probable that the parasites are migrating from the placenta

to develop elsewhere into crescents, because we do not find crescents in the peripheral blood. The failure of sexual forms to reach the peripheral blood must inevitably result in failure of the parasite to complete its development even when susceptible anophelines bite such women. The rare possibility of parasites of the schizogony cycle being transmitted congenitally to the child must be taken into consideration ; this would doubtless result in the formation later of gametes in the child, and so in a circuitous manner the stages infective for the mosquito would become available. In the meantime we have the fact, proved by abundant evidence, that the mere proliferation of *P. falciparum* on a colossal scale in one organ at least of native adult women does not result in the production of crescents in that organ, nor does it result in their appearance in the peripheral blood.

The anatomy and circulation of the placenta.

Before discussing the distribution of malaria parasites in the placenta, it is necessary to make same reference to the placental circulation. According to the descriptions and diagrammatic representations of the placenta and its circulation contained in many text books of anatomy, the condition is somewhat as shown in the left hand side of diagram No. 1. The arteries and vein of the child are carried from the umbilical cord, and pass subjacent to the amnion into the chorion ; the vessels are carried into the villous processes of the chorion ; the villi lie in the intervillous space and are bathed in maternal blood. As, however, the villi are covered by two layers of trophoblast, the cytotrophoblast layer next the chorionic process and the syncytiotrophoblast layer in contact with the maternal blood, the latter does not come directly in contact with the foetal blood vessels.

The maternal blood gains access to and leaves the intervillous space by arteries and veins which pass through the stratum spongiosum and the basal plate which represents the remains of the stratum compactum. The arteries as they enter the basal plate lose their muscular coat and they and the veins after this point consist of sinuous channels lined only by endothelium ; these channels open into the intervillous space, and at this point they lose their endothelial covering. The intervillous space is lined throughout by the syncytiotrophoblast layer. Therefore the walls of the

intervillous space and the villi which project into it are covered by the same lining structure.

The right-hand half of the figure represents the separated placenta; according to Gray and others this separation occurs through the stratum spongiosum. On the right half of the diagram have been shown the areas in which infected and uninfected red blood

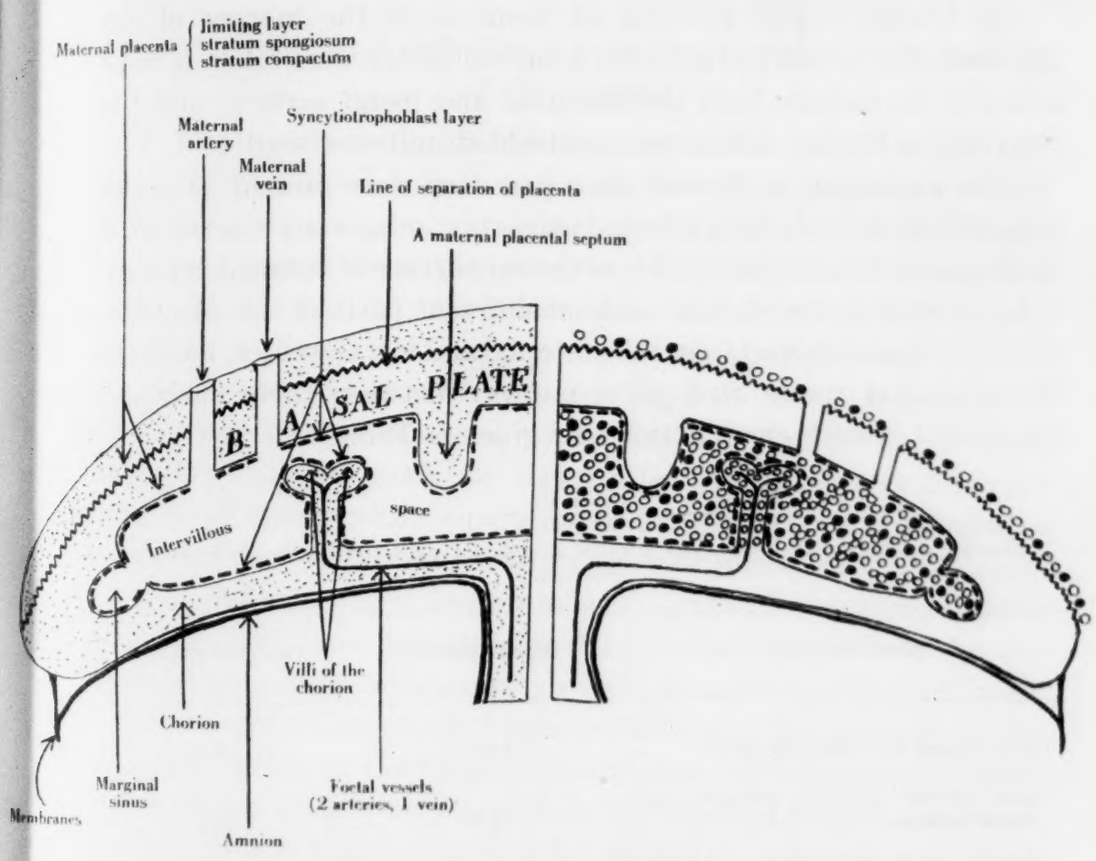


FIG. 1. Diagram of placental circulation (modified from Gray's anatomy). The left-hand figure represents the placenta before birth and the right-hand figure the same after birth. In the right-hand figure infected red cells are marked ● and uninfected red cells marked ○.

cells were found. The methods used were applied not only to the central portions of the placenta but also to the margin, and were :—

(1) The maternal surface of the placenta was carefully washed with normal saline solution ; smears were made from the surface, and also from blood obtained by scraping very lightly with a razor blade ; finally the thinnest possible slice of tissue was taken from the surface and from this thin slice films were spread.

(2) Films of blood from the placenta were examined at various depths from the maternal towards the foetal surface.

(3) The amnion was carefully washed with the normal saline solution and then reflected; from the surface thus exposed smears were made and then, as in the case of the maternal surface, a thin portion of the tissue was snipped off and smears made from this thin portion.

(4) Wedge-shaped portions of tissue from the margin of the placenta and cylindrical portions from the centre were taken in such a way as to include both the maternal and foetal surfaces and the intervening tissue; these were embedded and sectioned.

The examination showed that parasites were present in every preparation so made from infected placentas; some variation occurred as shown in the attached table, in the proportion of infected erythrocytes present in blood films made at different parts of the placenta, from the tissue snipped from the maternal and foetal surface, and from the placental tissue at a point midway between these surfaces; the results of such examinations are given in Table VI.

TABLE VI.

Showing percentage of erythrocytes infected at different parts and depths of the malarial placenta.

Blood films from	Edge of placenta	Centre of placenta
Tissue snipped from maternal surface ...	11·0	18·2
Tissue midway between maternal and foetal surfaces	9·8	36·2
Tissue snipped from foetal surface subjacent to amnion	8·2	9·4

As is seen in the table the centre of the placenta has a larger proportion of erythrocytes infected than the edge of the placenta; further, the portion situated midway between the maternal and foetal surfaces in the central portion is the most heavily infected of all.

We were unable to account for this unequal distribution in any way except on the assumption that infected cells tend to accumulate here while uninfected cells pass on. If this area presented a more suitable medium in which the parasites could complete sporulation,

we might expect to find a greater proportion of sporulating forms of parasite in the infected cells of this area. We enumerated the sporulating forms found in each area with the result that in all areas the percentage of sporulating forms was found to be approximately the same ; for example, in one case where 200 parasites were counted in each area by each observer, the percentage of sporulating forms in each area was approximately 14.

Consideration of the distribution of parasites in relation to the anatomy of the placenta.

A point which early attracted our attention was that although the peripheral blood of the mother was free from parasites not only at the time of labour, but also in individual cases which were followed for a month after labour, yet parasites could be found on the maternal surface of the placenta in large numbers. In sections of the placenta thin walled sinuses are found near the maternal surface, some of which, presumably maternal arteries, are free from parasites, while others, presumably maternal veins, contain numerous parasites. Assuming that the condition found after the placenta is delivered were in existence before separation of the placenta, it is difficult to avoid the conclusion that parasites must be present in the maternal veins of the placenta in large numbers. Possibly this is so, and the failure to find these parasites in the peripheral blood may be due solely to the peripheral immunity which we have already postulated.

We believe, however, that the distribution of the parasites on the maternal surface of the placenta found after delivery may not represent the distribution as it occurs in the placenta before separation. The placenta is comparable to a flat sponge on one surface of which is the relatively thick covering membrane composed of chorion internally, and amnion externally, while on the other is the much thinner membrane composed of the remains of the stratum spongiosum externally and the basal plate internally ; internal to these is the lining of trophoblast. The intervillous space with the villi projecting into it occupies the whole area between the maternal and foetal internal surfaces.

The intervillous space everywhere extends up to the maternal surface, as well as to the foetal surface, at the margin as well as in the centre of the placenta. Consequently infected erythrocytes

contained in the intervillous space lie right against the maternal and foetal lining of syncytiotrophoblast. The processes given off into the space from the foetal surface, namely the chorionic villi, have a counterpart in the more scanty septal processes into the space from the maternal basal plate. All these processes are covered

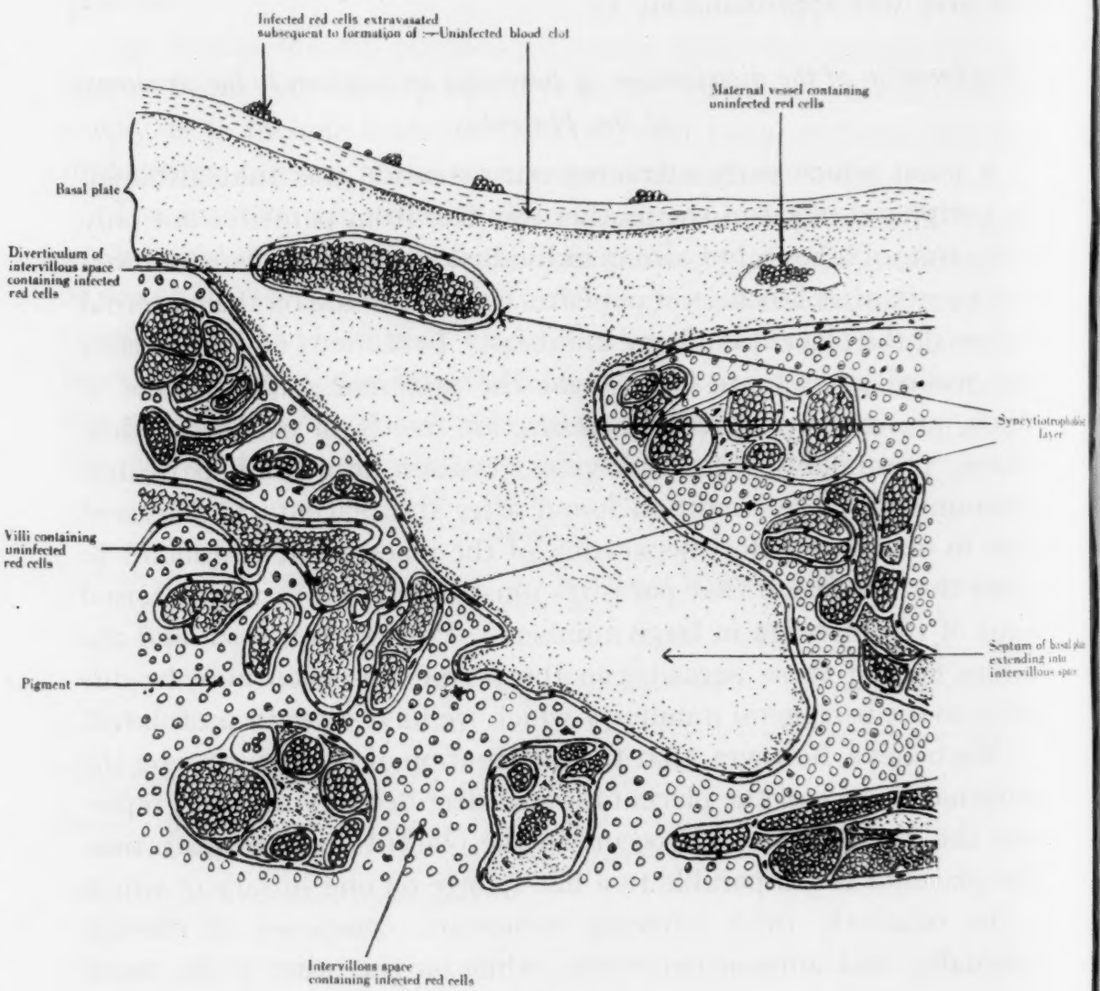


FIG. 2. Section through material surface of placenta [semidiagrammatic]. (Zeiss. Oc. 4. Obj. ½-inch.)

by syncytiotrophoblast and all are bathed in infected blood of the mother. At the origin of these processes are seen what at first appear to be areas of infected cells included in the chorion and basal plate. These are diverticula of the intervillous space cut across and are seen not only to contain infected erythrocytes

but also to be lined by syncytiotrophoblast and in some cases to contain a portion of uninfected villous process.

This arrangement in itself would suffice to explain the fact that films made from even the thinnest slice of tissue from the maternal surface or from the foetal surface after reflection of the amnion contain numerous parasites. The presence of infected erythrocytes on the uncut maternal surface of the placenta as seen after its delivery is probably brought about therefore by the aid of two factors, both of which are dependent upon mechanical compression by the uterus upon the placenta after expulsion of the child and separation of the placenta has begun. If the cord has been tied on the maternal side the uterus is contracting upon a mass of tense villi, out of which the blood cannot be expressed through the cord. As the uterine contractions continue and increase, and as the vessels of the villi remain rigidly distended with blood, diminution in volume of the placenta takes place in two ways following the line of least resistance. In the first place the maternal blood lying in the intervillous space is forced back through the remnants of the maternal arteries and veins and emerges on the maternal surface of the placenta. On further pressure the intervillous space ruptures where the membrane is thinnest, that is, through the diverticula on the maternal side, and liberates on to the surface its contained parasites.

We are inclined to believe that in normal circumstances parasites do not extend beyond the limits of the syncytiotrophoblast layer which lines the intervillous space and which invests all processes into it, whether villi of the chorion or septa from the basal plate. Parasites, indeed, as we have shown, may always be found close to the maternal and foetal surfaces and often penetrate into both, but in the latter case they are normally lying in sinuous prolongations of the intervillous space and are still contained within the limiting syncytiotrophoblast layer. We have noted that parasites have never been seen by us in the villi themselves.

Rupture of the diverticula of the intervillous space appears to be attributable primarily to ligation of the cord on the placental side. If the placental side of the cord were not tied there might still be backward oozing from the maternal vessels, but it is clear that this leakage and the leakage consequent upon rupture of the intervillous space diverticula are very much accentuated by the fact that ligation

of the cord keeps the villi which comprise a large proportion of the total volume of the placenta not only engorged with blood but practically incompressible. In some cases it is possible to see in section that the villi themselves are ruptured although this is relatively rare;*

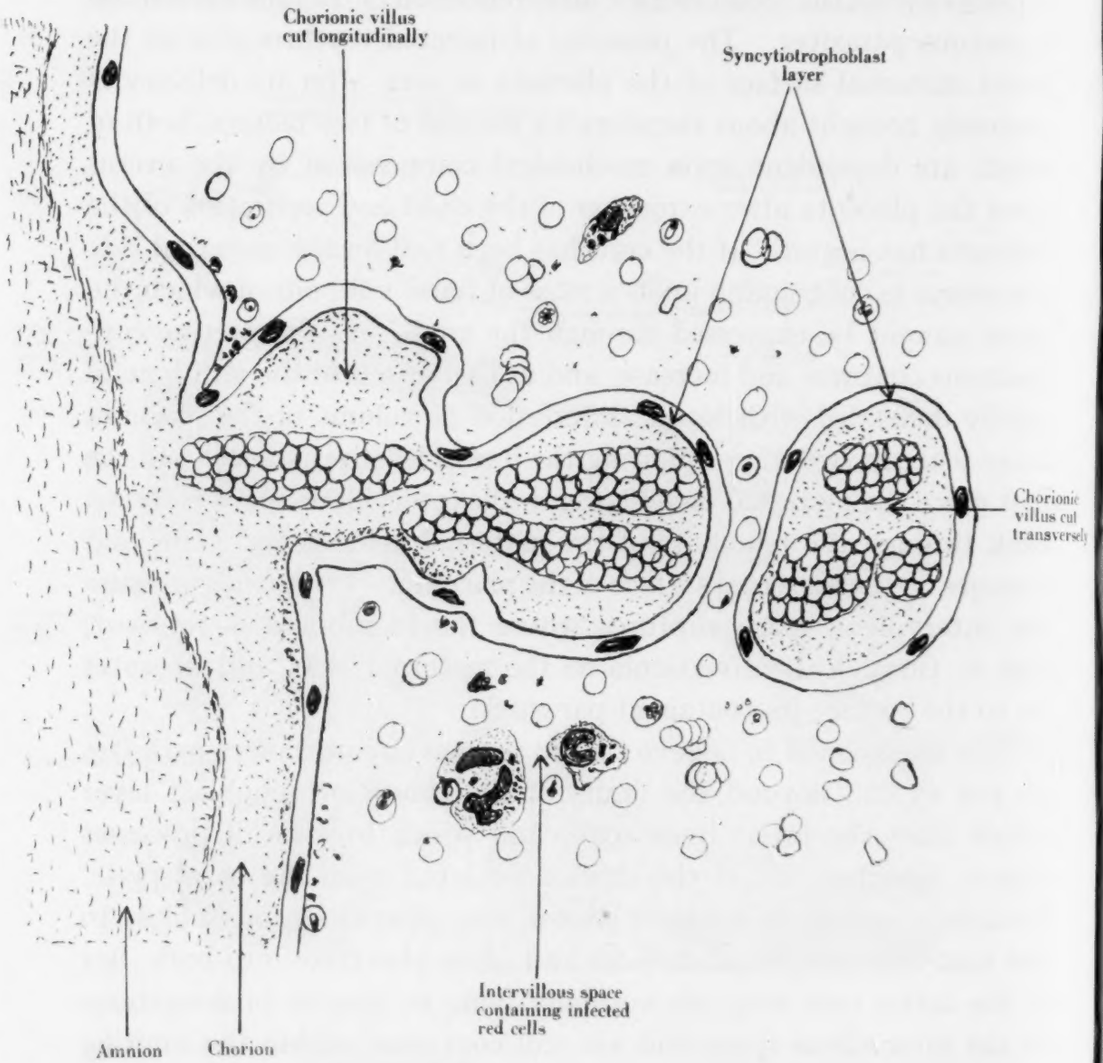


FIG. 3. Section through foetal surface of placenta, chorion and chorionic villi covered by syncytiotrophoblast layer [semidiagrammatic]. (Leitz. Oc. 0. Obj. $\frac{1}{12}$. Draw tube down.)

separation and rupture of the syncytiotrophoblast layer over the villus has been observed occasionally and, still more rarely, escape of uninfected red blood cells from the villus.

* Since the above was written, one of us (R.M.G.), has had the opportunity of comparing the sections of malaria-infected placentas with sections of a large number of normal placentas at the Coombe Hospital, Dublin; this comparison showed clearly that the vessels in the villi of infected placentas are greatly dilated, in some cases to such an extent that the chorion, which normally forms the main bulk of the villus, is almost obliterated. Presumably such villi are more liable to rupture. This condition is well shown in several of the villi in Fig. 2.

If the cord were not tied it is conceivable that such rupture of villi might result in infection of the child via the cord vein ; but seeing that rupture is such a rare occurrence even when the cord is tied on the placental side, that is to say, when we have shown the conditions are most favourable to injury to the villi, it seems highly improbable that rupture of the villi could occur unless the cord is tied.

If the cord is tied on the child's side and then cut, leaving the maternal end free to bleed, it is extremely unlikely that any villi could be ruptured. In any case even if they did rupture the infection in these circumstances would obviously not affect the child via the cord. Even when the end of the cord is tied the risk of rupture of villi is extremely small, and here again the child is not exposed to risk via the cord.

There is one aspect of the question, however, which, although it is in the nature of a side issue from our investigation, appears to deserve mention. The facts here brought out have a distinct application to a controversy which has frequently arisen as to the merits or demerits of tying the placental end of the cord. The most recent exponent of the view that such ligation of the cord is injurious, is Vaughan (1925). The procedure is condemned on the ground that ligation of the cord places the uterus at a disadvantage in its efforts to contract.

We have had in this series of malaria infected placentas a unique opportunity of studying this question, and have had a method of distinguishing sharply between the foetal and maternal bloods from the circumstance that the maternal blood contained such a high proportion of infected erythrocytes in many of the cases. The information obtained by us by means of the study of malaria placentas in which the cord has been tied on the placental side enables us to bring forward new evidence to support that school which claims that the uterus when contracting on villi with cord tied is contracting on a mass which for all practical purposes is incompressible. We are, however, far from saying that we believe that this is injurious to the uterus, or that it has any deleterious effect upon its power of expelling the placenta, or upon its own final involution. These matters are outside the scope of this investigation, but we believe that what we have described may stimulate their study on the part of those whose special province it is to deal with pregnancy and the puerperium.

V. CRESCENTS

It has long been recognised that crescents appear relatively rarely in the peripheral blood of adult natives in endemic areas. Recently, Christophers (1924) has confirmed Schüffner's view that crescent formation is not associated with immunization, but that on the contrary, crescent formation is reduced during the process. The negative findings as regards crescents in the peripheral blood and placentas in this series would be in accordance with the idea that the majority of these cases were so far immunized as to prevent the appearance of parasites in the peripheral blood, and that all were so far immunized as to prevent the appearance of crescents either in the peripheral blood or in the placenta. It is of interest in this connection to note that Horowitz-Wlassowa (1924), while noting a specific antibody present in most cases of malaria, has failed to demonstrate its presence in those cases which show many schizonts or gametocytes in the cutaneous blood.

In writing of the peripheral blood of children in endemic areas, Christophers says ' Crescents here are therefore associated with the higher values of parasites, and hence one may judge with the period of acute infestation rather than with that of immune infestation.'

The absence of crescents in the placentas of the native women discussed is clearly not due to lack of parasite proliferation ; it may be that the placenta is in all cases a site in which for some reason crescent formation does not occur ; as suggested in our previous paper, this might be due to some intrinsic and yet unknown character of this organ which renders it unfavourable to the development of mature sexual forms. On the other hand, it is possible that the failure of crescents to develop in the placenta is an indication of a certain degree of immunity having been reached. If this is so, it would indicate that we have in the placental absence of crescents an early sign of the development of immunity. We are then faced with a complex arrangement ; the patient is infected with *P. falciparum ;* there exists a degree of immunity which prevents the development of crescents in the placenta as shown by direct examination of this organ, and probably in other organs as shown by the failure to find crescents in the peripheral blood ; this anti-crescent immunity, then, would appear to be of a general nature affecting the whole circulatory system. Quite a different picture is presented when we examine the immunity against asexual parasites. While the

peripheral circulation appears to be immune with regard to them the placental circulation, far from being immune, offers a most suitable soil for their development on an immense scale.

It is important, first of all, to decide whether the absence of crescent formation which has throughout our series characterised the placental blood is due to an inherent character of all placentas. This could be done by examining the placentas of women who, although infected with malaria, have not resided sufficiently long in an endemic area to acquire any degree of immunity. This investigation could be carried out readily in Europe in the case of pregnant women who return after a short first residence in an endemic area, and who are infected with malaria. If it should prove that such women readily develop crescents in the placenta, it would be necessary to examine also natives of endemic areas who had gone to Europe, and who had infection of the placenta. If the latter cases still showed no crescent formation in the placenta, it would be reasonable to draw two conclusions, firstly, that the absence of crescent formation in the placenta in this series was not due to an inherent character of the placenta, and secondly, that it was due to an acquired immunity. The value of such an examination lies in the fact that if it can be shown that acquired immunity is the cause of crescent non-production, we have in the placenta an accessible internal organ which possesses very obvious advantages for the study of malaria immunity. In view of the difficulty of obtaining material here for the purpose of this study in non-immunized persons, this part of the work must be undertaken elsewhere.

It is frequently stated that crescents are produced more readily after quinine administration. Several of our series of cases had received quinine before labour, some of them for as long a period as ten days, yet in no case, as we have shown, were crescents found.

VI. PATHOLOGICAL EFFECTS OF PLACENTAL INFECTION WITH *P. FALCIPARUM* ON MOTHER OR CHILD

Before discussing any effects which might be attributed to malaria infection, we give an account of the material and examination upon which our conclusions are based. In spite of increasing efforts to obtain permission to examine material post mortem, we have still a vast amount of prejudice to overcome ; this can only be done by gradual education and awakening the interest of those most nearly

concerned, namely, the natives themselves. Our records of post mortem examinations are consequently rather meagre.

The following are the material examined and the methods adopted ; in all cases Leishman's or Giemsa's stain was used.

1. *Maternal peripheral blood.* Thin and thick films were examined from the blood of the ear, at the time of labour.

2. *Placenta.* This was usually examined within 6 hours after labour ; exceptionally as much as 24 hours elapsed before examination was possible. The surface of the placenta was washed and cauterised ; an incision was made through the cauterised area and blood from the bottom of the incision was taken up in a pipette and used for spreading films.

3. *Umbilical cord.* The cord after being washed and cauterised was cut in two places, one as near as possible to the placenta and the other about six inches from the placenta ; films were spread from the blood in the vessels.

4. *Peripheral blood of living children.* Immediately after the birth of the child, thick and thin films were made from the peripheral blood.

5. *Partial examinations of cadaver of dead born children.* In most cases it was impossible to obtain permission for a complete examination ; puncture of certain of the organs by means of a needle was permitted in some cases, in addition to examination of the peripheral blood.

6. *Complete examination of cadaver of dead born children.* Where permission could be obtained, a complete examination was made. This comprised smears of peripheral blood, liver, spleen, kidneys, bone marrow, lungs and other organs.

7. *Examination of cadavers of children who died within seven days.* The examinations carried out in these cases were of the same kind as those in number 5 and 6.

Material from post mortem examination of the children was preserved and material from the placentas was fixed, embedded in paraffin and sectioned.

A. Effect on the mother before and after labour.

In Table VII given below, the main facts concerning the 55 infected mothers and their children are set out, with special reference to the fate of the child.

TABLE VII.

Showing clinical history and peripheral blood findings in 55 mothers infected in the placenta with *P. falciparum*; also the fate of their children.

Case No.	Age	Peripheral blood of mother	Temperature 3 days before or after delivery	Quinine, total amount given before delivery	Child born alive	Child lived 7 days
1	20	+	105° F.	25 gr.	Yes	Yes
3	24	0	100° F.	0	Yes	Yes
4	21	0	105° F.	0	Yes	Yes
5	35	+	100° F.	0	Yes	Yes
6	36	...	100° F.	0	Yes	No
9	18	+	N	0	Yes	Yes
13	18	0	105° F.	30 gr.	Yes	No
16	22	+	103° F.	36 gr.	Yes	Yes
17	36	...	103° F.	5 gr.	No	...
19	30	...	...	0	Yes	Yes
20	26	0	N	...	Yes	Yes
23	22	0	102° F.	0	Yes	Yes
34	37	0	...	...	Yes	Yes
35	22	0	...	...	Yes	Yes
38	20	0	...	...	Yes	Yes
45	36	0	N	0	Yes (2)	No (2)
51	21	0	100° F.	0	Yes	Yes

TABLE VII—*Continued*

Case No.	Age	Peripheral blood of mother	Temperature 3 days before or after delivery	Quinine, total amount given before delivery	Child born alive	Child lived 7 days
52	22	o	N	...	Yes (1) No (1)	Yes ...
53	15	o	N	o	Yes	Yes
58	28	o	100° F.	o	Yes	No
59	22	+	N	o	Yes	Yes
63	29	+	N	o	Yes	Yes
64	24	o	100° F.	o	Yes (1) Yes (1)	Yes (1) No (1)
67	26	o	N	o	Yes	Yes
69	24	o	N	o	Yes	Yes
71	24	+	N	o	Yes	No
75	25	o	N	o	Yes	Yes
81	23	o	100° F.	o	Yes	Yes
86	21	o	N	o	Yes	Yes
92	22	o	...	...	No	...
93	23	o	N	o	Yes	Yes
94	20	o	100° F.	o	Yes	Yes
95	32	o	N	o	No	...
96	36	+	...	o	Yes	Yes
98	20	o	N	50 gr.	Yes	Yes
101	24	...	N	o	Yes	Yes

TABLE VII—*Continued*

Case No.	Age	Peripheral blood of mother	Temperature 3 days before or after delivery	Quinine, total amount given before delivery	Child born alive	Child lived 7 days
102	26	o	N	o	No	...
103	28	o	N	o	Yes	Yes
108	26	o	101·5° F.	o	Yes	No
110	28	...	N	o	No	...
118	30	...	102·5° F.	o	Yes	Yes
128	28	o	N	o	Yes (1) Yes (1)	No (1) No (1)
134	16	o	N	o	Yes	No
136	17	o	103·8° F.	o	Yes	Yes
137	18	o	101° F.	o	Yes	Yes
138	33	o	101° F.	o	Yes	No
140	26	o	104° F.	o	Yes (1) No (1)	No ...
142	20	o	N	o	Yes	Yes
143	22	o	N	o	Yes	Yes
146	34	+	100° F.	o	Yes	Yes
147	26	o	N	30 grs.	Yes	Yes
150	24	o	103° F.	o	No	...
151	25	o	N	10 grs.	Yes	Yes
153	17	+	102° F.	15 grs.	Yes	Yes
155	21	o	...	...	No (1) No (1)	... ...

As is shown in the table 23, that is, nearly fifty per cent. of the infected mothers had fever, and three cases in which fever is not recorded received 50, 30 and 10 grains of quinine. It will be observed that in two cases in which both peripheral and placental blood was infected, namely, cases 59 and 71, there was no fever ; these cases are of special interest as each presented a massive infection of the placenta. It is important to note that certain cases in which malaria was suspected to exist received quinine in varying amounts for different periods before labour without the placenta being cleared of parasites.* Conversely there are seven cases not shown in this table which were diagnosed on clinical grounds as malaria and received quinine in varying doses, and in which the placenta did not contain parasites at the time of birth. Although we know that quinine in doses quoted has failed to eradicate parasites from the placenta in the cases mentioned in the table, we cannot therefore justifiably assume that similar doses will fail in all cases. Some or all of these seven cases, had they not been treated, might have proved infected by placental examination.

In view of the number of cases who received quinine before labour and who still had infection in the placenta, it is possible that the doses were administered too late in pregnancy and in too small quantity owing to the fact that these cases only enter hospital when labour is imminent. It is known (Forchheimer (1915)), that quinine administered to the mother is excreted in the urine of the child. There were three deaths among the 150 mothers of whom the placenta was examined. None of the three who died showed malaria either in placenta nor in the peripheral blood. With regard to the post-partum history of the mothers, we have practically no information, as these cases make a very brief stay in hospital, seldom more than a week. It is therefore not possible to state whether these cases have recrudescences of malaria after leaving hospital. So far we have not had any opportunity of examining the placenta of a woman who at her previous labour had been proved to have an infected placenta.

That infected red blood cells are left behind in the uterus in large numbers when the infected placenta is born there is no doubt, for

* Since the above was written, we have had the opportunity of observing a case whose peripheral blood showed malignant tertian parasites a week before labour, and who subsequently received quinine grains 20 for six consecutive days. The placenta of this case showed a few parasites and many pigmented leucocytes.

we have already shown that parasites abound on the maternal surface of the infected placenta when born. We have also observed parasites in the blood which escapes during delivery of the infected placenta. In the process of delivery of such infected placentas this may be a source of danger where such blood is permitted to reach abrasions on the skin of the child or the attendant.

We must assume that after the expulsion of the main bulk of the parasites with the placenta, one of two events happens ; either the remaining parasites are prevented from entering the maternal circulation owing to closure of the uterine vessels, and they are then thrown out with the remains of the stratum spongiosum, or they are absorbed into the maternal circulation. In the former event the mother's peripheral and general circulation does not become infected from the placental source as a direct result of labour ; in the latter event the result would depend on two factors, i.e., the dose of parasites absorbed, and the degree of the immunity which exists in the peripheral and general circulation of the mother.

B. Effect on the children.

1. *Before birth.* A total number of 164 children were born of the 155 mothers, this figure includes premature children. The single births numbered 146, giving 146 children, and the twin births numbered 9, giving 18 children ; two was the maximum number produced at one birth. There were 148 children born alive and 16 born dead. In the case of children born alive, the only means at our disposal for ascertaining the transmission of malaria parasites or their products to the child *in utero* was the examination of the child's cord and peripheral bloods.

Of the total 148 children born alive 4 are omitted from consideration here because the placentas relating to them were not received or were in such a state of decomposition that they could not be examined satisfactorily. Of the remaining 144 there were 51, i.e., 35·4 per cent. who were born of mothers whose placenta was infected ; while 93, i.e., 64·6 per cent. were born of mothers whose placenta was not infected. Only a short examination period after birth was possible, rarely more than 7 days, but a few cases were observed for a longer period. Of the 51 children born alive, of mothers with infected placentas, 13, i.e., 25·5 per cent. died within 7 days, the

remaining 38 survived the observation period ; of the 93 children born alive of mothers whose placenta was not infected, 5, i.e., 5·4 per cent. died within 7 days, the remaining 88 survived the observation period. Of 18 children born alive, and who died within 7 days, 13, i.e., 72·2 per, cent. were born of mothers whose placenta was infected, while 5, i.e., 27·8 per cent. were born of mothers whose placenta was not infected. Of the 16 born dead, 2 are omitted because the placentas relating to them were in such a state of decomposition that they could not be examined satisfactorily. Of the remaining 14 there were 10, i.e., 71·4 per cent. who were born of mothers whose placenta was infected* and 4, i.e., 28·6 per cent. who were born of mothers whose placenta was not infected.

In none of the children born alive were parasites found at birth, either in the cord or peripheral blood ; nor were pigmented leucocytes seen in any of them.

In 22 cases of dead children, i.e., some of the 10 children born dead, and some of the 18 cases who died within 7 days, we had additional means of diagnosis by post-mortem examination, a partial examination in 12 cases and a complete examination in 10 cases.

None of the 22 children who were examined by one or other of the above methods presented parasitic infection in the peripheral or cord bloods, nor in any organ examined ; nor were pigmented leucocytes found in the cord or peripheral blood of any of them. In three cases, however, pigment was found either free or contained in leucocytes in the internal organs of the child ; the mother's placenta in each of these three cases was infected with malaria. We are not in a position to state definitely what the source and nature of this pigment are ; while it may probably result from red cell destruction in the child, there is no direct evidence to show that this red cell destruction was brought about by the malaria parasite invading the red cells of the child. Our completely negative findings as regards parasites in any of the children are opposed to the idea that the pigment was produced by the parasite itself acting on the child's blood cells. We cannot exclude the possibility of infection having existed in the child and having died out before the time of examination at birth. It appears possible that toxins of malaria absorbed from

* The only case in our whole series in which infection of the placenta was diagnosed by the finding of pigmented leucocytes without parasites being present, is included in this group.

the focus of infection in the placenta will produce red cell changes in the child. In case 64 binovular twins were born with cords attached to adjacent placentas ; these placentas and cords presented remarkable differences in the blood as shown in Table VIII.

TABLE VIII

Showing the differences in the placentas, cords, and fate of the child in twins (both placentas infected).

	Placenta		Cord		Child	
	A	*B*	*A*	*B*	*A*	*B*
Macroscopic appearance of placenta, and fate of child	Anaemic	Normal	Anaemic in first 6 inches only	Normal throughout	Died within 15 hours	Lived 7 days +
Type of parasite ...	30 % of parasites sporulating	No sporulating form seen	...	...	...	...
Number of parasites	1-15 fields	1-25 fields	...	...	...	...
Microscopical appearances	Evidence of great destruction of red cells	Normal except for infected red cells	Same as placenta for first 6 inches, remainder normal	Normal	Normal	Normal

The degree and stage of infection in the two placentas was different ; *A* having a higher degree of infection and a high proportion of sporulating forms. The uninfected red blood cells of placenta *A* presented all stages of lysis ; poikilocytosis and anisocytosis were present. Cabot's rings, pseudospirochaetes and fragmented red cells were very numerous ; on the other hand the uninfected cells of placenta *B* appeared normal.

In cord *A*, apart from the absence of parasites, the changes in the blood were identical with those seen in placenta *A* ; the changes noted were, however, confined to the first six inches of the cord nearest the placenta ; beyond this point cord *A* appeared normal ; cord *B* was normal throughout its length. The peripheral blood of both children appeared to be normal in so far as the non-nucleated red

cells were concerned, and a differential count of the leucocytes, and a comparison of the nucleated red cells showed the following results.

Peripheral Blood Diff. Leuc. Count	P.	LM.	SM.	Eos.	Bas.	Nuc. Red.
Twin *A*	39·0	8·5	40·0	1·5	...	11·0
Twin *B*	51·5	13·0	22·5	4·0	0·5	8·5

The different appearances of the blood in placentas *A* and *B* are illustrated in figures 3 and 4 in the Plate. This case is of interest in that whereas no parasites were found in the child and cord *A*, yet there was evidence in the cord blood of extensive damage to red cells similar to the damage in the placenta *A* blood.

We are compelled to leave unanswered the question what exactly is the pigment found in the organs of the child, in the cases referred to. It is suggestive, however, that in three cases in which pigment was found in the organs of the child, in each case it had died ***in utero***, and that there were marked changes in the blood of the placenta belonging to each child; these changes resemble closely the appearances found in the placenta *A* and the first position of cord *A*; in case 64 no pigment was found. The remarkable appearance of the blood in a small portion of cord *A*, i.e., that nearest the placenta, and the similarity of the appearance in the blood of this part of the cord and that of the corresponding placenta suggest strongly that some agency acting in the placenta in causing destruction of red cells had also acted on the blood of the child in the portion nearest the placenta at the time the cord was tied. This agency we suggest is toxin liberated by the parasites sporulating in the placenta. If this child's cord had not been tied for some time after sporulation had occurred in the placenta, it is probable that all trace of this extensive localised destruction of red cells in the cord would have disappeared, being carried away by the circulating blood. The toxin which had in this case begun to pass into the child's blood stream was confined and prevented from circulating by the ligature of the cord, and so was acting in a concentrated form on a limited amount of blood with the results noted and illustrated. The toxic effects were

not observed in the blood of the cord at any point further away than six inches from the placenta.

In Table IX we give a summary of the salient facts concerning the children born dead or who died within 7 days, and in Table X which follows this, we give the figures which would represent the expected results in these cases if we assume that malaria had no part in the production of the mortality.

TABLE IX

Showing the number and percentage of children who were born dead or who died within 7 days, among 61 children born of 55 infected mothers and 97 children born of 95 uninfected mothers.

	Total	Born of 55 infected mothers		Born of 95 uninfected mothers	
		Number	Percentage	Number	Percentage
Children born dead	14	10	71·4	4	28·6
Children who died within 7 days ...	18	13	72·2	5	27·8
Totals	32	23	71·9	9	28·1

In Table X below are given the figures which would be expected provided that malaria infection had no influence.

TABLE X

Showing the totals and percentages in each group in Table IX redistributed in proportion corresponding to the ratio of the infected to the uninfected mothers, i.e., 55 infected to 95 uninfected in a total of 150 cases.

	Total	Born of 55 infected mothers		Born of 95 uninfected mothers	
		Number	Percentage	Number	Percentage
Children born dead	14	5·1	36·4	8·9	63·6
Children who died within 7 days ...	18	6·6	36·7	11·4	63·3
Totals	32	11·7	36·6	20·3	63·4

From a consideration of these two tables we can conclude, if such a small group can be taken as representative, that malaria here has a definite and important effect in the production of a high proportion of infant deaths *in utero* and in the first week of life.

It is difficult to say whether any isolated group of figures is representative of the true facts among a large population, but when it is remembered that these cases here discussed comprise the vast majority of all cases treated in the maternity hospital, and that they include members of every important tribe living in Freetown, we may legitimately assume that they form a fair sample of the urban population in this endemic area.

We believe that almost conclusive evidence is provided by the figures considered above ; but over and above this we have obtained from the study of a large number of infected and uninfected placentas data which convince us that the pathological alterations in the malaria-infected placenta are such that they cannot fail to have a deleterious effect on the child in one or more ways.

(1) *Congenital malaria.* In spite of the enormous infection seen in many placentas we have not seen any parasitic evidence of this condition. We are, therefore, in a position to repeat for this larger series of cases what we said in our previous account of our first 26 cases, namely, that this condition is of great rarity. In view of this it is interesting to note that in other countries very different results have been obtained ; for example, Ziemann (1924) records that Weselko, in 1922, in Albania attributed to congenital malaria the death in the first week of 144 children of mothers infected with *P. falciparum,* while Swellengrebel (1925) records 48 cases of congenital malaria in the near East in each of which a microscopical diagnosis was made at periods varying in time from 1 to 5 days after birth.

(2) *Interference with the nutrition of the child.* We have shown that in some cases as many as 65 per cent. of the red blood cells in the intervillous space are infected. It appears certain that in so far as the red cells are concerned in the nutrition of the child their function must be very seriously interfered with.

(3) *Toxic effects.* It is evident that large amounts of malaria toxins are being produced in heavily infected placentas. It is possible that the toxins produced by the malaria parasite are, as Ziemann (1924)

suggests, anchored in the maternal tissues, or they may be incapable of reaching the foetal circulation ; but the similarity of the blood changes observed in many infected placentas and in the placental portion of the cord in such instances as case 64 quoted above lead us to suppose that the toxins are at least in some cases capable of penetrating into the child's circulation. It appears probable from the above facts that definite effects on the child are brought about by the two last factors, that is to say, interference with nutrition and toxic absorption.

VII. THE AGE INCIDENCE OF MALARIA

(1) *Mothers.* The age incidence of 55 cases of malaria occurring among 148 mothers is shown in Table XI.

TABLE XI

Showing the distribution according to age of 148 maternity cases and of 55 placental malaria infections amongst them.

Years	Total maternity cases	Total malaria cases	Percentage infected
15-20	32	12	37·5
21-25	55	20	36·4
26-30	37	13	35·1
31-35	8	5	62·5
36-40	14	5	35·7
41-45	2	0	...
Totals	148	55	37·2

Excluding the 41-45 age period in which the figures are exceptionally small, the general effect of the table is to show that parturient women at all ages are equally susceptible to malaria infection in the placenta. Taking this table in conjunction with Table VII it will be observed, in so far as clinical manifestations of malaria and effect on the children are concerned, that there is no outstanding difference between the ages groups. It is surprising to observe that some very young mothers, age-group 15-20, e.g., case 53 age 15, and case 9

aged 18 in Table VII with placenta infected can not only pass through labour without clinical manifestations of malaria infection, but can also give birth to apparently perfectly normal children. That such tolerance exists only in some cases is, however, exemplified by case 13 aged 18, and case 153 aged 17.

(2) *In adult males and adult non-parturient females.* The figures we have in these groups have already been mentioned, i.e., adult males 150 and adult non-parturient females 43. As the total infection in these groups as judged by examination of the placental blood was only 2 per cent. and 7 per cent., respectively, a curve plotted from them would yield little information as regards the age incidence.

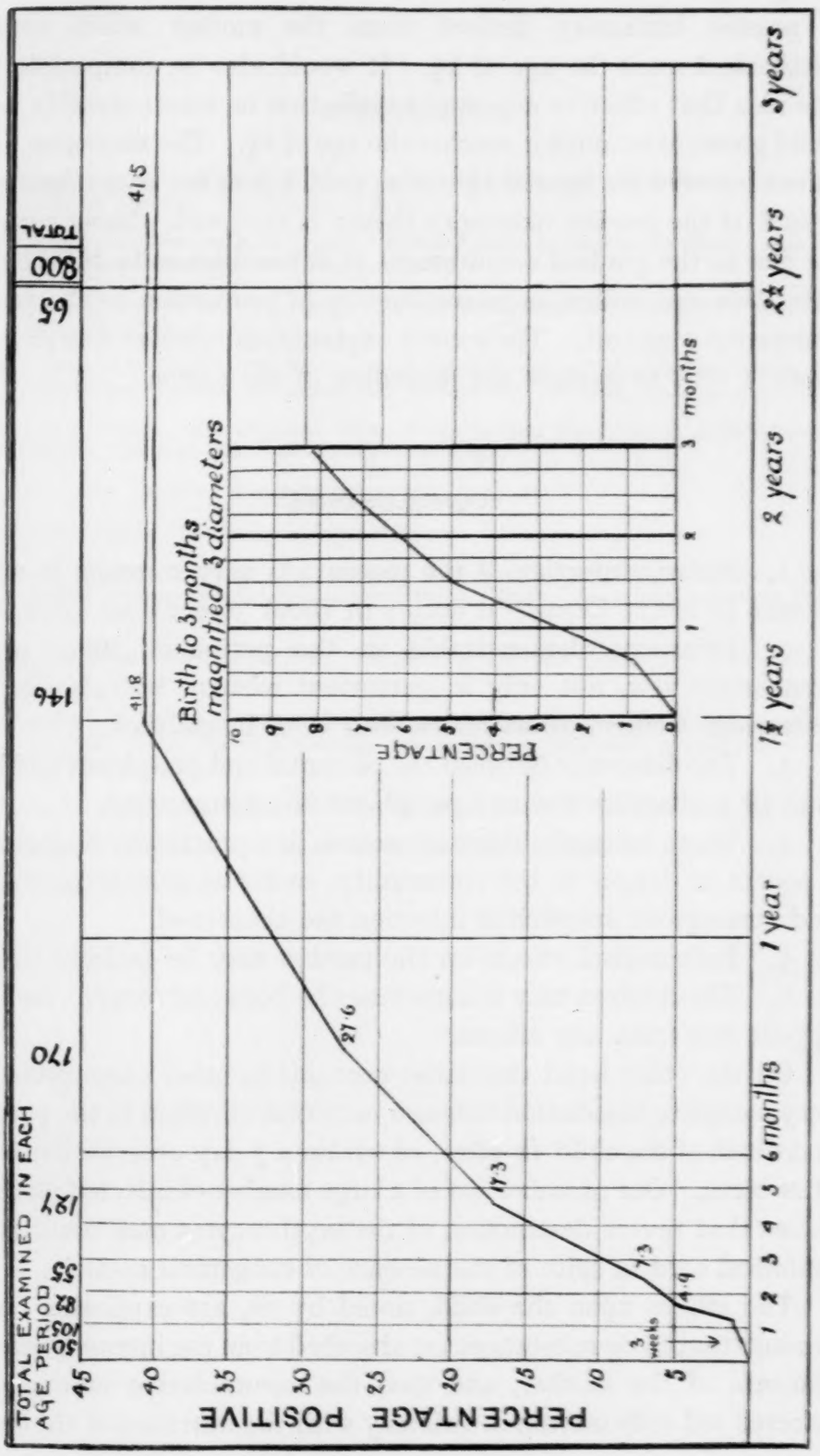
(3) *In children.* The peripheral blood of 158 children born of 150 mothers of whom 55 were infected in the placenta, and 95 were not so infected, proved negative as regards malaria at birth. In addition we have examined with negative results the peripheral blood of 41 new born children of 36 mothers ; the placentas of these mothers were not examined for malaria, but in 35 of them the peripheral blood was examined with the result that two were found positive, diagnosed by the finding of pigmented leucocytes. We were unable to follow the progress of these 199 hospital cases in order to ascertain when they would become infected. The only information to be derived from them in this connection is that at birth and for a period of a week or so after birth no infection was found in any of them. From an infant clinic, however, we are able to provide the figures already dealt with in Table II from which we can show the age distribution of malaria among 800 young children, namely a series of children of ages up to 2½ years.* In each case of this series the peripheral blood was examined once. In Graph II is shown the distribution according to age of infected cases among these 800 children examined, the examinations extending over a period of a complete year.

It is seen that only one case is recorded as positive during the first month of life. From this time on to the age of 1½ years the infection in children shows a regular rise. The character of this curve could be explained in either of two ways. It would be

* Nine children under 2½ years who appeared in the total for seasonal incidence cannot be included here on account of uncertainty as to their exact age.

GRAPH II

Showing distribution according to age of the malaria infected cases among 800 children at their first appearance.



compatible with the idea that the child at birth was endowed with a passive immunity derived from the mother which steadily diminished until the age of 1½. It would also be compatible with the idea that effective exposure to infection increases steadily as the child grows older until it reaches the age of 1½. The flattening of the curve between the ages of 1½ and 2½ (which is as far as our figures go) would, if the passive immunity theory is accepted, almost certainly be due to the gradual acquirement of active immunity by the child after this age, which increases directly in proportion as the passive immunity wears off. The second explanation appears less probable since it fails to explain the flattening of the curve.

CONCLUSIONS

1. Malaria infection of the placenta is very common in native women in Sierra Leone; it occurs in about 36 per cent. of cases.

2. Infections demonstrable in the peripheral blood are in comparison few, not only in parturient women, but also in non-parturient women, adult males, and even in children.

3. The difference between the placental and peripheral incidence is in all probability due to a peripheral blood immunity.

4. These intensely infected women are practically negligible as a source of danger to the community, so far as gamete production and consequent anopheline infection are concerned.

5. Pathological effects on the mother may be entirely lacking.

6. The children may in some cases be born and survive, and may appear free from any effects.

On the other hand this series contains in other cases proof of a very complete association between maternal infection in the placenta and death of the child *in utero*, or within a 7 day observation period after birth. Our examination of a large number of infected placentas shows that severe destruction of the erythrocytes may occur in the umbilical cord in spite of the absence of congenital malaria.

The effects upon the child, noted by us, are explicable on the grounds that toxic substances are absorbed from the intensely infected placenta of the mother, and that the accumulation of masses of infected red cells interferes seriously with the nutrition of the foetus.

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EXPLANATION OF PLATE VI

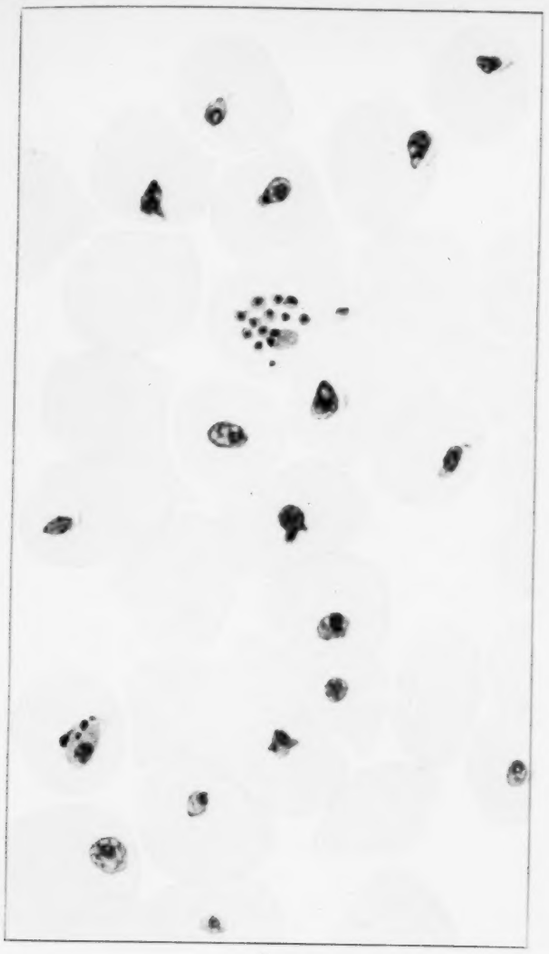
P. falciparum in the placenta.

Fig. 1 Microscopic field from an intensely infected placenta.

Fig. 2. Intensely pigmented type of placenta. Drawn from the tail of the film.

Fig. 3. Case 64. Placenta A.

Fig. 4. Case 64. Placenta B.



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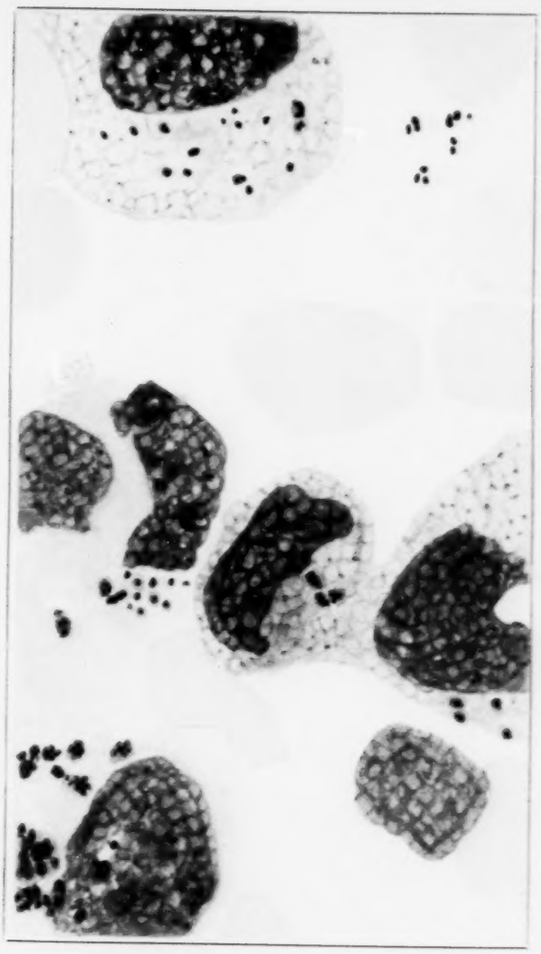

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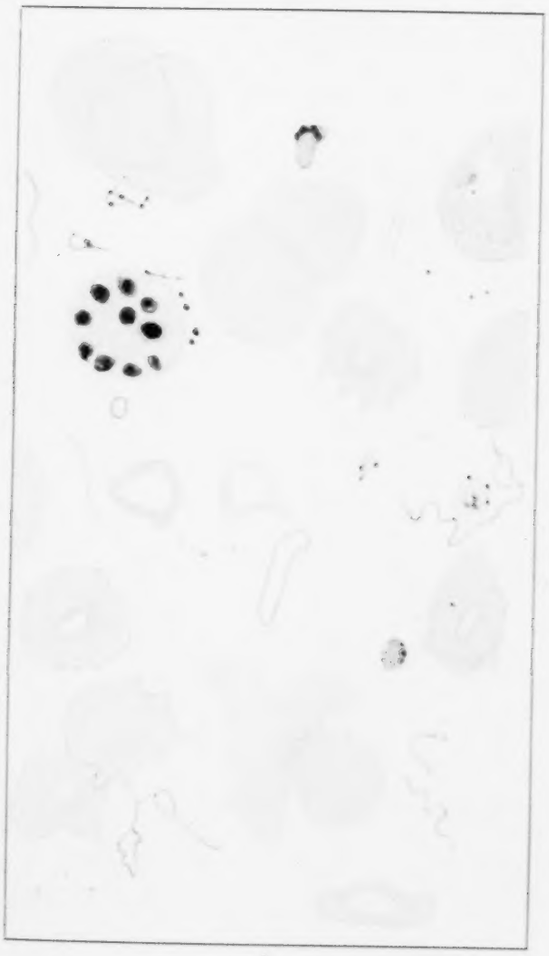

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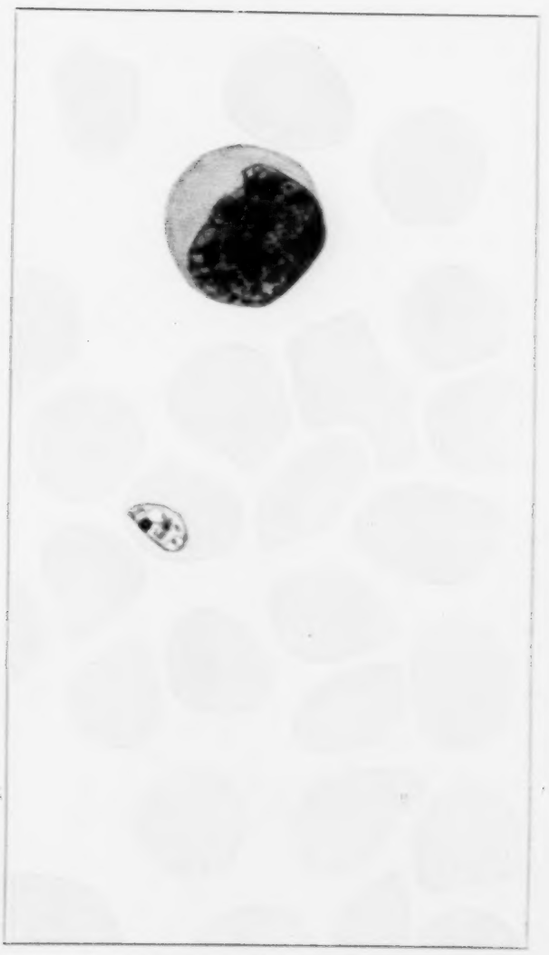

4.

THE EXPERIMENTAL TRANSMISSION OF CUTANEOUS LEISHMANIASIS TO MAN FROM *PHLEBOTOMUS PAPATASII*

BY

S. ADLER

AND

O. THEODOR

MICROBIOLOGICAL INSTITUTE, JERUSALEM

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Sandflies have been suspected by various authors of being the transmitting agent of oriental sore. The first definite evidence in favour of the *Phlebotomus* theory of cutaneous Leishmaniasis was provided by Wenyon (1912) who found about six per cent. of the sandflies he dissected in Aleppo infected with *Herpetomonas* resembling cultural forms of Leishmania. Wenyon states that all intermediate stages of development between the small non-flagellated bodies and the fully developed flagellates occurred. Later Mackie (1914) found ten per cent. of *Phlebotomus minutus* in Assam infected with *Herpetomonas* but Patton (1922) has pointed out that oriental sore is not endemic in Assam. In Mesopotamia where oriental sore is common and sandflies are a pest, Patton (1919) states that *Herpetomonas* is present in *Phlebotomus papatasii* and *P. minutus*, and in 1922 the same author remarks on the presence of *Herpetomonas* in *Phlebotomus papatasii* and *P. minutus* in Palestine.

Additional evidence for the *Phlebotomus* theory was adduced by Acton (1919) who showed that the distribution of oriental sores on the body corresponds to the distribution of bites of *Phlebotomus*.

In Palestine the epidemological evidence for the *Phlebotomus* theory of transmission of oriental sores (Jericho Boils) is ambiguous but rather favourable to the *Phlebotomus* theory. Canaan (1916) who first demonstrated Leishman-Donovan bodies in oriental sores from Jericho considered that town to be the only endemic centre of cutaneous Leishmaniasis in Palestine. Later however Kligler (1923) reported three cases from Kantara, Dostrowsky (1925) described ten cases from Artuf and also found one case from Bethlehem and one from Mozza, a small village near Jerusalem.

The aetiology of the disease as described by Dostrowsky in Artuf is of great interest. The population of Artuf is one hundred and fourteen and until 1923 sandflies were not observed in Artuf according to the statement of Dr. E. Jaruslawsky, the local physician (sandflies when present in numbers never pass unobserved). In the summer of 1923 the insects had for the first time become a pest to the villagers. This fact raised Dr. Dostrowsky's suspicion that the *Phlebotomus* was the carrier of oriental sores.

In June, 1924, one of us (A.) on Dr. Dostrowsky's suggestion examined the village and found every house infested with *Phlebotomus*. Three species *P. papatasii, P. minutus* and *P. perniciosus* were present, *P. papatasii* being by far the commonest. Dr. Dostrowsky then examined the population (ninety-seven) of an Arab village only one hundred metres from Artuf. Material was taken from every suspicious papule and given to one of us (A.). On examination Leishman-Donovan bodies were absent in every case. An examination of this village made by one of us (A.) did not reveal a single specimen of *Phlebotomus*. This may be explained by the fact that this village consists of mud huts without windows and uniformly dark in the interior, while the first village consists of fairly modern houses with whitewashed interiors, the furniture and clothes producing shade and contrast to the whitewashed walls. In Jerusalem, Jericho and Artuf it was noted that *Phlebotomus* prefers the shade produced by contrast to uniform darkness.

In Jericho *Phlebotomus papatasii* and *P. minutus* occur in large numbers, the former species predominating. *P. perniciosus* is absent or very rare. In Mozza *P. papatasii* and *P. perniciosus* are both common. It would seem then that *P. papatasii* is the carrier of cutaneous Leishmaniasis in Palestine, it being the only species common to three localities where cutaneous Leishmaniasis occurs. Schroetter, (1923), incriminated an insect which he called *Phlebotomus el Ghor* as the carrier in Jericho but Martini has shown that this insect is not a *Phlebotomus* and is incapable of biting.

Against the *Phlebotomus* theory is the fact that cutaneous Leishmaniasis is absent from many localities in Palestine where sandflies are a plague, e.g., the village of Rehoboth containing twelve hundred inhabitants where all three Palestinian species

of *Phlebotomus* abound. In Haifa (population about 30,000) which is the place most infested with sandflies in the whole of Palestine, all three Palestinian species being present, oriental sore is hitherto unknown. Still more striking is the fact that up to the present no locally acquired cases of cutaneous Leishmaniasis have been noted in Jerusalem itself. There are always a number of cases of cutaneous Leishmaniasis in Jerusalem from Jericho, Bagdad, Aleppo and Persia. In addition *Phlebotomus papatasii* is very common, *P. perniciosus* also occurs, and *P. minutus* is very rare, i.e., there are, according to the *Phlebotomus* theory, ideal conditions for the spread of the disease. Were even a small number of cases present they would not pass unnoticed for all classes of the population of Jerusalem assiduously attend the numerous clinics of the city for even the most trivial maladies and physicians are on the look-out for a case of locally acquired oriental sore.

It would seem then that on the assumption that a *Phlebotomus* sp. is the carrier of the disease in nature a third and hitherto unknown factor apart from human cases and insect carriers is necessary for the spread of the disease. What this factor is still remains to be investigated.

EXPERIMENTAL TRANSMISSION TO HUMAN BEINGS

Sergent, Parrot, Donatien and Béguet (1921) first described the experimental transmission of oriental sore to a human being. These authors divided five hundred and fifty-nine sandflies into twenty-three batches, crushed them in saline and used the resulting material for inoculation into the arms of twenty-three volunteers. The material was collected at Biskra, an endemic centre of oriental sore, and the experiments were performed at Algiers where oriental sore is unknown.

Only one experiment from a batch of seven specimens of *Phlebotomus papatasii* gave a positive result. The experiment was performed on the 20th August, 1921; two months and twenty days later a papule was noted, and on the following day numerous Leishman-Donovan bodies were found in the papule.

In October, 1924, one of us (A.) commenced an examination of *Phlebotomus* in Jericho. Two hundred and twenty specimens of

Phlebotomus from Jericho, of which one hundred and seventy-four were females, were dissected during October to December, 1924, and three females gorged with mammalian blood were found to contain *Herpetomonas* in their midgut. All stages from non-flagellated forms to long flagellated forms were noted.

The following is a method recommended for the examination of *Phlebotomus* for *Herpetomonas*. If the insect contains no blood, cut off the terminal abdominal segment, stroke the upper surface of the abdomen with a needle to push out the ova, gently pull the head with one needle, holding the other needle against the upper surface of the thorax. In this way the head, oesophagus, salivary glands, oesophageal diverticulum and midgut are removed together, and the individual parts of the alimentary tract can be examined for *Herpetomonas*. If the midgut contains blood gently pull the head away from the thorax. The salivary glands, oesophagus, and oesophageal diverticulum and occasionally the upper part of the midgut will come away together with the head. The rest of the alimentary canal can then be pulled out from the hind end in the usual way.

In December, 1924, sandflies became very rare in Jericho and on the 20th December, 1924, a search through the whole town by a trained assistant yielded only four specimens of *P. papatasii*. Subsequent monthly examinations revealed no sandflies until April 20th, 1925. They were not found on April 4th, 1925, but on April 20th, 1925, they were numerous, *Phlebotomus papatasii* only being present. *Phlebotomus minutus* appeared towards the end of June.

A batch of one hundred and ninety-eight sandflies of which one hundred and ninety-one were *P. papatasii* (one hundred and seventy-five females and sixteen males) and seven *P. minutus* (six females and one male) were collected in Jericho on the 25th June, 1925, and brought to Jerusalem for dissection. Of this batch only one specimen, a female *P. papatasii*, was found to contain *Herpetomonas*. The insect contained no trace of blood and the abdominal cavity was full of ripe or almost ripe eggs. The whole alimentary tract was found to be swarming with *Herpetomonas*. Flagellates were found in the oesophagus, oesophageal diverticulum, midgut and hindgut. They were especially numerous in the upper part of the midgut where swarms of parasites appeared to be attached to the posterior surface of the oesophageal valve, so much so that some parasites appeared at first sight on examination in the fresh preparation to be intracellular. (The oesophageal valve is a well-marked structure in *Phlebotomus*.)

The fact that flagellates were noted in the oesphageal diverticulum is of great interest for in freshly dissected specimens it is frequently seen that waves of peristalsis pass from the posterior end of the oesophageal diverticulum towards the oesophagus, which in *Phlebotomus* is very short, so that the oesophageal diverticulum practically opens into the pharynx. It is thus possible that flagellates may be propelled into the pharynx and buccal cavity and the possibility of a direct infection by the bite of a sandfly must be considered. Hitherto it has been generally held that infection takes place only through the crushing of an infective *Phlebotomus* on the skin, a theory which is very feasible in view of the large number of sandflies crushed on the skin (e.g. Cornwall, 1922). Another point of great interest was observed, i.e. the flagellates were not polymorphic as in the cases observed by Wenyon (1912) and by one of us (A.) in 1924, but they were all elongated and had long flagella. The oesophagus, together with the piece of oesophageal diverticulum and the upper part of the midgut, containing the oesophageal valve, were dissected away together, and another part of the midgut behind the oesophageal valve was dissected off separately; these parts were placed on separate slides, fixed in absolute alcohol, stained overnight with Giemsa, differentiated with a 0·02 per cent. solution of acetic acid and permanently mounted.

The remainder of the material was used for inoculation into the forearm of a volunteer. The volunteer had previously been exposed for three years (1917-1920) to oriental sore in Mesopotamia without contracting the disease. Two points on the skin of the left forearm were scarified and material containing flagellates was rubbed into each on the 26th of June, 1925. On the 31st July, 1925, a small papule which would normally have passed unobserved was noted on one of the inoculated points and on examination Leishman-Donovan bodies were found. The incubation period was thus less than half that noted by the Sergents and their collaborators (1921).

The dissection of individual sandflies and experimenting with material from one infected individual is more satisfactory than crushing large numbers in saline and experimenting with the product, for in the latter case it is impossible to know whether a negative result is due to the fact that none of the sandflies contained

Herpetomonas or whether the *Herpetomonas* was non-infective. From April to June, 1925, a thousand and thirty-seven sandflies collected in Jericho were dissected and found negative for *Herpetomonas ;* on the 9th of June another two hundred sandflies from Jericho were collected and on dissection found negative. Thus the experiment of crushing more than twice the number of sandflies from an endemic centre used by the Sergents and their collaborators and inoculating a single volunteer would have given a negative result.

Nothing was noted on the other site of inoculation; nevertheless the part was scraped and on the 31st of July, 1925, examined for Leishman-Donovan bodies with a negative result. It was again examined on the 4th of August, 1925, with a negative result.

The successful results of the above experiment and the experiment of the Sergents and Parrot, Donatien and Béguet prove that human beings can be infected with oriental sore by inoculation with *Herpetomonas* from *Phlebotomus papatasii*, but the epidemiological evidence that *P. papatasii* is the only carrier of the disease in nature is not yet complete.

In view of the successful infection with Leishmaniform parasites by injection of *Herpetomonas ctenocephali* into mice, rats, and a dog, and by injection of *Crithidia fasciculata* into mice and rats (Laveran and Franchini, 1913), by injection of *Herpetomonas jaculum* from the water scorpion *Nepa cinerea* into mice, by feeding a puppy on dog fleas (Fantham and Porter, 1915), by injecting *Herpetomonas jaculum* and *H. culicis* into birds (Fantham and Porter, 1915) the possibility of other sources of infection apart from *P. papatasii* must be considered in spite of the fact that the distribution of sandflies corresponds more closely than that of any other biting insect to the distribution of oriental sore. It must be pointed out, however, that among others Hoare (1921) working with *Crithidia melophagia*, *Herpetomonas jaculum* and *H. calliphorae* on mice, sticklebacks, newts and frogs, and Shortt (1923) working with *Herpetomonas ctenocephali* and *H. lucilae*, failed to infect monkeys, dogs, rabbits, rats, mice, pigeons and frogs.

The question of other insect carriers of oriental sore apart from *Phlebotomus papatasii* can only be cleared up in the future by direct experiments on human skin with flagellates from various insects.

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CO-ORDINATION OF EFFORT IN TSETSE-FLY INVESTIGATIONS

(A paper read at the second Imperial Entomological Conference, London, 15 June, 1925)

BY

WARRINGTON YORKE

(Received for publication 9 September, 1925)

I have been asked by the Administration of Northern Rhodesia, which I have the honour of representing at this Conference, to put before you the case for co-ordination of effort by the various African Colonies in Tsetse-fly investigation, principally in the experimental determination of the Game-fly relationship. It gives me considerable satisfaction to open a discussion on this subject, as I have long felt the necessity for co-operation of effort if any definite advance of knowledge is to be achieved. In fact, it will be within the recollection of certain here present that I pressed this point of view at the first Imperial Entomological Conference, five years ago, and at a subsequent meeting of the Royal Society of Tropical Medicine, when certain recommendations of the Glossina Sub-Committee of the Bureau of Entomology came up for review.

Now let me make it clear at once that nothing which I shall say to-day is in anyway directed against entomological research. As I said five years ago, I do not wish to place difficulties in its way, but I desire most sincerely to encourage it; and have the greatest admiration for the work which is being done by the various entomologists scattered throughout Tropical Africa, who, at considerable risk to themselves, are doing their utmost to advance knowledge. But I have long held, and I see no reason to change my views, that the problem is not a purely entomological question and that, in devising any plan of research, we must bear this in mind. The real problem is, of course, Trypanosomiasis of man and his domestic animals, and it comprises four factors: (1) the

pathogenic virus or trypanosomes; (2) the population and the domestic stock; (3) the transmitting agent or tsetse-fly, and (4) the reservoir of the virus or big game. In my judgment substantial advance in knowledge can only be achieved by research carefully devised and adequately co-ordinated with the object of taking into consideration, at the same time and in the same locality, all of the above factors. In short, I advocate centralization of effort.

What has been done in the way of research into the Trypanosomiasis problem since the Glossina Sub-Committee of the Imperial Bureau of Entomology published its report five years ago? Probably only those who, like myself, have to read and summarise the innumerable papers dealing directly and indirectly with this subject are in a position to realise the enormous amount of human energy which is being devoted to it. There are the entomological papers of Lloyd, Swynnerton, Carpenter, Fiske, Schwetz and others; the very able and suggestive epidemiological papers of Duke; the dozens of papers dealing with the action of various drugs on infected man and stock, not to mention equally numerous papers of a purely academic nature. These reports are scattered throughout a vast range of journals and periodicals. I can assure you the mere task of reading and summarising them for the *Tropical Diseases Bulletin* is no small undertaking. While the reviewer is filled with admiration for the energy and enthusiasm shown by these reports, he is only too conscious of the fact that the energy is often misdirected, that the reports, although often very long, frequently, owing to the omission of some essential information, do not permit of any inference, that even work admirably conceived and executed is often brought to nought by the fact that those who are conducting it go on leave without arrangements being made to carry it on. As an illustration of this one cannot do better than cite his own experience in attempting to summarise the position of knowledge regarding the therapeutic action of certain drugs in trypanosomiasis. Time and again one finds a record of most carefully conducted observations on long series of patients; then at periods varying from a few months to a year after the commencement of the work the observer goes on leave, or is placed on some other duty, and nothing more is heard of the patients. Now we can only judge of the result of treatment by ascertaining what has happened after the lapse of a

number of years, and as this information is in the vast majority of instances not forthcoming, the initial excellent work is thus rendered useless and knowledge or, shall I say, ignorance, remains *in statu quo*.

I had a letter the other day from Dr. Lloyd, who, as you know, has wide experience of tsetse work and is at present investigating the subject in Nigeria. He tells me that he has fenced round a small area with the object of making a game exclusion experiment. He writes :—

'The fence was less trouble to construct than I anticipated, and is strong enough to keep out small stuff, but would not stop a roan or buffalo. In construction, when it was about three-parts done, a herd of some thirty roan got in and were there when we went to work. The labourers started to chase them and did capture a small one, but the great beasts crashed and leaped through in twenty places and the noise frightened some pig at the pool and they drove the wire out pig-shape in a dozen spots. After it was closed it took some clearing, although the area is only a half square mile. Duiker were the worst trouble as they got into the thickets and would not be flushed. However, it is clear of antelope now. The results are promising to be of interest but I fear they will not be convincing. *Tachinoides* does not seem to be affected but *morsitans* has become very scarce compared to the control. As an anomaly the infection has gone up considerably. This shows that most of, if not all, the *morsitans* in the place are emigrants from the neighbouring belts of fly. The main point of interest is the very emaciated condition of the female flies.'

Well, gentlemen, I know Dr. Lloyd intimately, and was associated with him on the Luangwa Commission. He is an extremely able and conscientious worker and, if his foreboding should unfortunately turn out to be true and the results prove unconvincing, it will, I am satisfied, not be through any fault of his, but merely of the system under which he is working. As most of you know I have always been an ardent advocate of a large and carefully controlled experiment of game destruction in a localised area and believe that from it we should obtain information of the greatest possible value. Some of you will be relieved to hear that it is not my intention to enlarge upon this much-debated subject on the present occasion. I do not like to assume the rôle of a prophet, but I am afraid that we shall not learn much from Dr. Lloyd's experiment. He is evidently too short of funds to carry it out efficiently and on a sufficiently large scale ; he is working almost single-handed and it is very doubtful to me whether, if unassisted, he will be able to make all the observations that such an experiment demands. Finally, in order to obtain information of real value from an experiment of this sort, not only

must it be preceded by a thorough and scientific investigation of the conditions, both in respect of fly and of the trypanosomiasis of man, stock and game, but it must be followed by an equally careful investigation extended over a sufficient length of time—probably running into a number of years—or no precise information regarding the results of game elimination can be expected. In due course Dr. Lloyd will, doubtless, go on leave and then, if we can be guided by what usually happens on such occasions, the work will either come to an end, or, which amounts to the same thing, someone who has other interests, or no interests at all, will take over.

In connection with this game exclusion experiment, you will perhaps pardon me if I refer to a passage in the extremely interesting Report of the East Africa Commission which I had the pleasure of reading a few weeks ago. The passage runs as follows :—

> ' The question of game destruction is a very thorny one and has aroused much feeling. In this connection the opinion of Mr. Walter, now Lord, Rothschild, is worth recording : " To prove to the utilitarians the absolute uselessness of this proceeding, I should like to point out that the extermination of the game animals in any large area would be a task of several years' duration and the following would take place. As, year by year, the large animals grew scarcer, the tsetse flies *Glossina palpalis* and *morsitans*, which are the means of spreading sleeping-sickness in man and nagana in animals, would be driven to bite monkeys, carnivora, rats, mice, and the numerous small animals of those regions ; these would be infected and the trypanosomes of the disease would gaily survive. This would not only mean the continuance of the disease in its present degree, but would also cause a sharp increase of both diseases." '

Now, sir, I must confess that personally I should have experienced some difficulty in finding anything less worth recording. In the first place, Lord Rothschild ventures to prejudge in the most categorical manner, and without the slightest evidence, what would happen as the result of an experiment—to my mind a most dangerous and unwarrantable procedure—and in the second place, even assuming his premise, for which of course we have similarly no support, namely, that tsetse flies in the absence of large animals would be forced to feed on monkeys, carnivora, rodents and mice, the inference that ' these would be infected and the trypanosomes of the disease would gaily survive, and that this would not only mean the continuance of the disease in its present degree, but would also cause a sharp increase of both diseases,' indicates complete

ignorance of what is known regarding the effect of the pathogenic trypanosomes of man and stock on these small animals.

I mention this because it illustrates in such an admirable manner my point that the Trypanosomiasis problem is not one which can be fully investigated by entomologists alone or, for that matter, by any other class of worker.

It is not my intention to refer in detail to the most valuable work which Dr. Duke is carrying out in Uganda on the protozoology and epidemiology of the disease. All who are familiar with his work realize its great value, but here again I feel that the work is suffering because Dr. Duke's other duties make great demands upon his time and because he lacks sufficient expert assistance and adequate financial resources to put to the crucial test the theories which he has built up at the cost of years of patient research. I am glad to learn that one of the recommendations of the International Conference on Sleeping Sickness which met in London last month under the auspices of the 'League of Nations' is that the International Commission, which it is suggested to form, should be placed under the presidence and control of Dr. Duke, and I am especially glad to see that they have coupled the recommendation with the suggestion that Dr. Duke's staff should be increased by the services of a biochemist and entomologist. Apart from this, I do not hope for much from the labour of the International Conference. Such a Conference seems to me to be premature. I cannot believe that its efforts are likely to advance knowledge and we hardly know enough at the present time to formulate regulations governing the International Frontiers in Tropical Africa. In my judgment, much more is to be hoped from an inter-Colonial Conference and from the co-ordinated and sustained effort which it would be in the power of such a Conference to ensure.

The case for co-ordination appears to me to be overwhelmingly strong, for the following reasons :—

Many investigators are at present working in more or less isolation at the different aspects—entomological, epidemiological, pathological, and therapeutic—of trypanosomiasis. The cost of this work is divided amongst many Colonies and therefore probably does not fall unduly heavily upon any one Colony, but in the aggregate the total annual expenditure must be very large. Unfortunately, as the

individual workers are isolated, insufficiently supplied with funds and assistance, and compelled to leave their work at more or less stated intervals, and sometimes at periods which are not stated, it is not surprising that much that is done is unsatisfactory and incomplete owing to want of organisation to ensure continuity, and consequently much time and money is wasted. Such a process has continued long enough; it is uneconomical and although the expense to each Colony may be relatively slight, in the aggregate it is large, and knowledge, if it advances at all, does so slowly and uncertainly.

Many of the problems which demand solution are very large, as, for example, the relationship of game to trypanosomiasis and the tsetse or the various problems which, five years ago, the Glossina Sub-Committee proposed should be investigated. Such problems cannot, with any hope of success, be investigated by isolated workers, whether entomologists or pathologists, but only by large and well-equipped Commissions having at command large funds.

I would therefore urge, as I did five years ago, (1) that in future, effort should be concentrated instead of dissipated; (2) that the work of the entomological and medical and veterinary research into the Trypanosomiasis problem be combined under one central organisation, and such organisation be supported by pooled contributions of all the African Colonies interested; (3) that the personnel of the investigating commission or commissions be large enough to ensure continuity of work in all directions, thus obviating interruptions due to such exigencies as illness or leave and preventing staleness and inertia, which is so likely to result from isolation; (4) that sufficient funds be placed at the disposal of the investigating commissions to allow of the employment of adequate native labour, so that experimental work can be undertaken on a sufficiently large scale, thus enabling the investigation of the relationship of fly and trypanosomiasis to game, and of the various problems enumerated in the Report of the Glossina Sub-Committee of the Imperial Bureau of Entomology, to be carried out in a satisfactory manner and with some reasonable prospect of success.

Whether such a scheme would cost more than is at present being spent individually by the different Colonies, I do not know. Certainly a large sum would be needed, and if this were not forthcoming the whole plan would collapse. Whether the problem is sufficiently

grave to warrant a large expenditure in a serious endeavour to find a solution, I will not attempt to discuss, as I am neither a politician nor a student of political economy, but judging from the valuable Report of the East Africa Commission to which I have already referred I gather that certain politicians and economists are abundantly satisfied of the gravity of the situation.

A NOTE ON MICROFILARIAE IN TANGANYIKA TERRITORY

BY

J. F. CORSON

(*Received for publication*, 15 *August*, 1925)

PREVIOUS OBSERVATIONS

Geographical distribution and incidence. Little appears to be known of endemic areas. Feldmann (1904), Marshall (1909) and Grothusen (1910) noted the prevalence of *Mf. perstans* in the district of Bukoba. It had been observed previously by Zupitsa, in 1897-98. Feldmann examined over 6,000 persons and found from 24 to 86

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Page 319. For 'recommended by a Committee of the Royal Institute of Public Health in 1903' read 'recommended by a Committee of the Royal Institute of Public Health in 1914.'

Page 360. For 'examination of the placental blood' read 'examination of the peripheral blood.'

(1920) found 32.32 per cent. of 297 native soldiers at Dar-es-Salaam infected with *Mf. bancrofti*. They, however, like the population of the coast towns, include people from many different parts.

Incidence in Dar-es-Salaam. In the years 1908-09 and 1909-10, microfilariae were found in slightly over 2 per cent. of nearly 40,000 thick blood films, examined chiefly for malaria and taken, no doubt, in the day-time. During the years 1922, 1923 and 1924, in the course of routine examination of a large number of thick blood films at the bacteriological laboratory at Dar-es-Salaam, microfilariae,

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PREVIOUS OBSERVATIONS

Geographical distribution and incidence. Little appears to be known of endemic areas. Feldmann (1904), Marshall (1909) and Grothusen (1910) noted the prevalence of *Mf. perstans* in the district of Bukoba. It had been observed previously by Zupitsa, in 1897-98. Feldmann examined over 6,000 persons and found from 24 to 86 per cent. of the population, in various parts of the district, infected, the northern parts showing a greater incidence than the southern. In the south-east of the territory the district around Liwale appears also to be an endemic area as *Mf. perstans* was found by Mr. Irvine, sub-assistant surgeon, in 1924, in 31·5 per cent. of 402 adults examined. In regard to *Filaria bancrofti* even less is recorded. Elephantiasis was stated by Grothusen (1909), and previously by others, to be endemic in the Ubena-Ebene area, to the north-west of Lake Nyassa. Recent reports point to the prevalence of this disease in districts near the great lakes, as Mwanza and Bukoba in the north, and near Tukuyu (Neu Langenburg) in the south. In the region of the sea coast, there appear to be many cases of elephantiasis in Mafia Island, though no figures are yet available, while a considerable number are operated upon in the larger towns. Engeland (1920) found 32·32 per cent. of 297 native soldiers at Dar-es-Salaam infected with *Mf. bancrofti*. They, however, like the population of the coast towns, include people from many different parts.

Incidence in Dar-es-Salaam. In the years 1908-09 and 1909-10, microfilariae were found in slightly over 2 per cent. of nearly 40,000 thick blood films, examined chiefly for malaria and taken, no doubt, in the day-time. During the years 1922, 1923 and 1924, in the course of routine examination of a large number of thick blood films at the bacteriological laboratory at Dar-es-Salaam, microfilariae,

sheathed and unsheathed, grouped together, were found, as recorded in the annual reports, in 2.5 per cent., 2.2 per cent., and 3.6 per cent., respectively. The much greater incidence of *Mf. bancrofti* than of *Mf. perstans* in such examinations, was noted by Engeland and Manteufel (1911).

Species of microfilaria found. *Mf. bancrofti* and *Mf. perstans* only were found by Engeland and Manteufel. Fülleborn also (1908 and 1913a) found only these two species, with one possible exception, in blood slides received from Bukoba, Usumbura and Shirati, near the great lakes in the north, and from Dar-es-Salaam. The possible exception was a very small sheathed microfilaria, found by Manteufel (1911) in the blood of a soldier's boy in Dar-es-Salaam. The slide sent to Fülleborn, broken on the way, contained only about 10 microfilariae, stained with haemalum. Fülleborn admitted, with all reserve, that it might belong to a new species or be *Mf. powelli*, but that its possible identity with *Mf. bancrofti* could not be excluded. *Mf. loa* has not been found. Neave (1912) gives a list of flies found in German East Africa, which includes three species of Chrysops, viz., *C. bicolor* Cordier, *C. longicornis* Macq, and *C. magnifica* Austen. Limited reference only to subsequent literature has been available and this may not represent present knowledge. It is of interest that a considerable number of natives of West Africa were brought to this country during the late war.

Periodicity of Mf. bancrofti. That well-marked periodicity occurs in at least a great majority of cases was shown by Engeland. He found the microfilaria, as stated above, in 32.32 per cent. of soldiers, in the night blood, but in only 2.06 per cent. were they present also in the day time. Whether or not forms without periodicity also occur, as in the Southern Pacific and, perhaps, in West Africa, has not apparently been shown.

PRESENT OBSERVATIONS

From 300 to 400 thick blood films, chiefly of Africans, but including some Indians, taken at various unstated hours of the day, are examined monthly in ordinary routine work at the bacteriological laboratory at Dar-es-Salaam. In 768 such films, examined recently, microfilariae were found in 6.7 per cent. The latter figure refers to different individuals so the percentage given is a minimum.

Similar films were taken between 10 and 11 a.m., from 140 school pupils and from 140 prisoners. In the former, *Mf. bancrofti* was found in eight cases (5·7 per cent.) and *Mf. perstans* in one. In the latter *Mf. bancrofti* was present in seven cases (5 per cent.) and *Mf. perstans* in five. Examination in detail, including measurements with a camera lucida, was made in 30 cases, and observations as to periodicity in 21 cases. In a few cases only were living specimens examined and 'vital' staining with neutral red and azur II used. Staining with weak methylene blue, as described by Foley (1913) and by Sharp (1923) was not employed. Giemsa's stain and haemalum were chiefly used. The results confirm those of German observers. Microfilariae indistinguishable from *Mf. bancrofti* and *Mf. perstans* were the only kinds observed. Brief mention of a few details only is therefore made.

Mf. bancrofti. Morphology. The *sheath*, in nearly all the specimens, appeared to be unstriated. In one specimen the free anterior part of the sheath, in length about equal to one-fourth that of the worm, showed very clear, regular cross striation. If, as has been suggested by Fülleborn (1913b), it may be simply an impression of the striation of the body of the worm, in this case it was remarkably well-defined. Striation of the sheath has been observed by Brumpt (1922), who suggested that it might indicate a larval skin, and by Foley. It is referred to in a review of a paper by Biglieri (1923). The *anterior end* of the worm, in specimens stained with Giemsa's solution, showed various appearances, corresponding more or less with the descriptions of various authors. An interpretation of them could not confidently be made. Neither in living nor in stained specimens could a 'fang' or 'prepuce' be recognised. *Striation of the body*, in deeply-stained specimens, was observed to extend throughout the whole length of the worm, from the extremity of the anterior end to the tip of the tail. With ordinary staining it was not seen in front of the first nuclei. The *nuclei* were counted in a few specimens. The results did not agree with the figures given by Sharp. The number in front of the 'nerve ring,' for example, was from 60 to 70 or more. The appearances of the '*excretory cell*,' the '*central viscus*' or '*Innenkörper*' and the 'G^1 *cell*' of Rodenwaldt agreed with Fülleborn's description and illustrations of *Mf. bancrofti*. The *tail* was not infrequently folded upon itself.

Measurements. 114 specimens from 26 cases. As the tip of the tail could not be seen clearly in many specimens, the *last tail nucleus* is assumed to be at 95 per cent. of the total length. The average position in 17 specimens measured was at 94.6 per cent.

The terms denoting the ' fixed points ' of Fülleborn are used.

N.	Ex-P.	Ex-C.	G^1-C.	L. Tail-C.	Total length.
19	29	29.5	69.7	272.8	(287.1)

In over 83 per cent. of 114 specimens the ' *nerve ring* ' was situated at a point between 18 per cent. and 20 per cent. of the length. The following are the figures of Fülleborn's measurements of 28 examples.

Microfilariae from German East Africa ; ordinary thick dry preparations from 5 different slides ; haemalum staining.

	N.	Ex-P.	G^1-C.	A-P.	L. Tail-C.	Total length
Average ...	19.9	30.1	70.3	82.6	95.3	263.7
Minimum ...	18.3	27	67	79 ?	94.4	245.5
Maximum ...	21.1	33	73.3	88.4?	96	291

Periodicity. Only persons in whose blood microfilariae were found in the daytime were examined. Measured quantities, viz., 20 c.mm. of blood were taken in 13 cases. In eight other cases, by the kindness of the staff of the native hospital, two thick films at midnight and two by day were taken on two or three successive days. The day time was usually 9 a.m. In no case was the number of microfilariae in the day blood equal to that in the night and in nearly all cases the disproportion was greater than could be accounted for by ordinary fluctuations, at the same hour on successive occasions, of, say, 300 per cent. Fluctuations are known to be considerable. In one case, for example, the number in 20 c.mm. of blood taken at the same hour of the night on several occasions, varied between 235 and 604. This case showed 17 at 5 p.m. and one only at noon, on two occasions. The greatest number found in daytime blood was 62, in 20 c.mm. of blood at 9 a.m. In this case, at 11 p.m., the number was 211. One case showed seven at noon and 13 at 11 p.m. So far as any conclusion can be drawn from these few observations, it would appear that a form of *Mf. bancrofti* without periodicity, if existing at all in this country, must be rare.

Mf. perstans. Citrated blood was centrifuged and dehaemoglobinised, for examination, especially of the anterior end.

In living specimens a small spot, usually terminal, occasionally apparently lateral, was seen. 'Vital staining' with neutral red showed a small conical structure, base to the front, just behind the outline of the anterior end. In some specimens the anterior margin appeared to show minute papillae. No constant feature, however, could be seen in the examination of about 20 specimens. No 'fang' was seen.

Stained specimens. These showed the morphological characters of *Mf. perstans.* The nuclear 'break' at 84 per cent. was a fairly constant character. Deep staining failed to show more than very slightly-marked cross striation.

Measurements. 47 specimens were measured. There is some lack of uniformity in measurements given by different authors even in the position of such a constant and sharply-defined feature as the 'nerve ring.' The following examples are given for comparison.

Brumpt (1922). Fülleborn's terms are used.

N.		Ex-P.		A-P.
26.4	...	36	...	83

These figures are quoted by Stephens and Yorke (1921).

Rousseau (1919).

N.		Ex-P.		G^1-C.		A-P.
25	...	32	...	62.6	...	83.5

Macfie and Corson (1922).

N.		Ex-P.		G^1-C.		A-P.
22.5	...	32.7	...	62.3	...	81.1

In the present cases the average figures were as follows :—

N.		Ex-P.		A-P.		Total length.
22.8	...	31.9	...	84.2	...	189.2

In over 90 per cent. of the specimens the position of the 'nerve ring' was between 20 per cent. and 24 per cent. of the length.

The small form of *Mf. perstans* was not observed.

SUMMARY

1. Little is known of endemic areas of filarial infection in Tanganyika Territory. In addition to districts near the great lakes in the north-west, *A. perstans* is probably endemic in the south-east, around Liwale.

2. *Mf. bancrofti* and *Mf. perstans* are the only forms known to occur.

3. *Mf. bancrofti* was found to have a well-marked periodicity in all cases in which sufficient numbers were present, though occurring, in a small proportion of cases, in the blood in the daytime.

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SPRING RELAPSES IN BENIGN TERTIAN MALARIA

BY

ADAM PATRICK, M.D., M.R.C.P.

PROFESSOR OF MEDICINE, UNIVERSITY OF ST. ANDREWS.

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While engaged, between 1916 and 1918, in the treatment of soldiers who suffered from malaria and who had been invalided from the Salonika army to the hospital base at Malta, I was struck by two clinical facts. The first was that patients who had been transferred to convalescent camp after recovery from attacks of benign tertian malaria, showed an extraordinary tendency to have relapses in the early spring, although the weather was genial; the second, that several patients whom I had treated (1919) in the late autumn, for severe subtertian malaria, remained well for two months or longer, and then relapsed about February, but this time with a benign tertian infection. It is possible that the men referred to in the latter case had previously experienced attacks of this form of malaria, although, in some instances at least, there was no history of it. It seemed possible that the tertian infection remained latent for some months after inoculation of the subject, and showed itself for the first time some months later. Indigenous malaria is extremely rare in Malta, and one could be all but certain that the tertian infection had not been acquired on the island, but there was no certainty that men coming from such a highly malarious country as Macedonia had not previously suffered from a benign tertian attack.

Acton, Curjel and Dewey (1921) record a similar experience. Of a series of 102 malignant tertian infections received for treatment at Dagshai, in India, in 1918, only seven were diagnosed as mixed parasitic infections due to the benign and malignant tertian parasites; yet in this series there were 64 benign tertian relapses, indicating that the majority of these mixed parasitic infections had been overlooked.

Some time ago a patient of my own developed benign tertian malaria in a manner which leaves little doubt that the first attack of the disease occurred after an incubation period of several months.

W.A., aged 37, a ship's officer, consulted me on 2nd December, 1921, about an illness which had been diagnosed as malaria. His ship had been in Bombay for a fortnight, from 1st October, 1921, and just before she left port he fell ill with malaise and headache, but without rigor or vomiting. This illness ceased in three or four days. A fortnight later, on the voyage to Europe, he had a second attack, of four days' duration, with similar symptoms. A third attack occurred about 7th November, while his ship was lying at Antwerp, and then for the first time he had shivering and some vomiting. When I saw him four weeks later, he felt out of sorts, but had 'on days and off days.' The spleen was considerably enlarged, and subtertian parasites, both rings and crescents, were present in the blood.

He was given a thorough course of quinine, beginning with a daily intravenous injection of bihydrochloride for four days, the first of 10 grains, and the three others of 15 grains each. From 7th till 26th December he took, by the mouth, 30 grains of quinine sulphate daily in solution ; thereafter, for a month, 20 grains a day ; and then 12 grains daily. A few crescents were present in the blood on 9th December. On seven subsequent examinations, up to 10th February, no parasites were found. By the middle of January the patient felt very well, and remained well, still taking 12 grains of quinine sulphate daily, until 8th March, when he vomited in the evening, complained of headache, and shivered a little. Two days later he had a more severe attack, and a two-day periodicity was established when I was called by his doctor to see him on 15th March. On that date the spleen was two inches below the ribs, and parasites, now benign tertian, were numerous in the blood. Subtertian forms were not observed, and the patient said that the symptoms were quite different from those which he had previously experienced, the shivering and headache being severe, and the attacks periodic. He quickly improved with a further course of treatment, but relapsed on 15th June, when benign tertian parasites were again found in the blood. There was a further relapse at the end of September, after

which he remained well until he passed out of my observation in January, 1923.

In this case there was no history of previous benign tertian malaria, and as the period from December till March was spent in the west of Scotland, infection must have taken place at least five months before the first attack. His previous visit, before October, 1921, to a malarious country was in March of the same year, when his ship had called at Bombay. But his first malarial attack of any kind did not occur till October, 1921. The subtertian parasites were apparently killed off by the course of quinine, but the benign tertian resisted it and caused an attack, even although the patient was taking 12 grains of quinine sulphate a day. It is to be noted that this tertian attack came on in the spring, as in the cases I referred to in the opening paragraph.

Although no reference is made to this peculiarity of tertian malaria in most text-books, the point is not a new one, for Dr. J. G. Jack has drawn my attention to a passage in Shakespeare's *Henry V*, Part 1, Act 4, Scene 1, lines 111-112, where Hotspur says :

> ' No more, no more ; worse than the sun in March,
> This praise doth nourish agues.'

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ANTI-RABIC PROCEDURE IN PALESTINE WITH SPECIAL REFERENCE TO DECENTRALIZATION OF TREATMENT

BY

G. STUART, M.A., M.B., Director

AND

K. S. KRIKORIAN, M.D., Assistant Senior Medical Officer

Laboratory Section, Department of Health, Government of Palestine

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I. INTRODUCTION

Rabies has been known to be endemic in Palestine for very many years. The country, in virtue of its geographical position, is one peculiarly liable to the disease in that it is bounded on three sides by countries in which rabies is prevalent while its general configuration favours the continued existence of the common 'carriers' of the disease—the jackal and the pariah dog.

In the decade immediately prior to 1923 in Palestine, the most convenient course open to persons bitten by animals suspected of rabies was to proceed to Jerusalem to attend for treatment at a private institute where Pasteur's original dried cord method was practised.

On the establishment of railway communications between Palestine and Egypt in 1918, however, the Army Authorities made the decision that all military personnel affected should be sent to the Anti-Rabic Institute in Cairo.

This somewhat unsatisfactory state of affairs continued until it became increasingly apparent that the necessity for the provision in Palestine itself of a mode of treatment, recognized as adequate both by Military and Civil Authorities alike, was absolute, if numbers of lives were not to be sacrificed every year. Such loss of life was ascribed to two causes: to lack of proper facilities for treatment in this country, and to ineffective treatment in Cairo consequent upon the lateness of arrival of the patients.

Early in 1923 the serious attention of the Department of Health was given to coping with the situation ; there was no doubt that persons in ever-increasing numbers were being bitten by rabid dogs and jackals all over Palestine ; the result was that an undertaking to supply an anti-rabic vaccine of undoubted reputation and of proved reliability,—one able to fulfil all army and civilian requirements—was given by the Laboratory Section of the Department.

Table I shows the number of untreated cases reported on as having died in hospitals during 1922 from Hydrophobia, and well illustrates the gravity of the situation at that time.

TABLE I.

No. of cases	Sex	Age	Wounds (all inflicted through naked skin and not cauterized)			Biting animal	In days	
			No.	Location	Gravity		Period of incubation of hydrophobia	Duration of symptoms
1	M	13	1	Outer surface lower leg	Slight	Dog	38	4
2	M	13	1	Finger	Slight	Dog	22	2
3	M	78	3	Dorsum of hand	Severe	Dog	44	4
4	M	47	3	Eyebrow, Nose, Upper Lip	Severe	Jackal	16	3
5	M	22	2	Hand	Slight	Dog	30	3
6	M	12	2	Ala and tip of nose	Medium	Dog	17	1
7	M	8	1	Tip of nose	Medium	Dog	44	2
8	M	45	2	Nose	Medium	Jackal	32	4

Additional considerations, however, emphasised the necessity for the undertaking, not the least amongst which was the fact that all expenses in connection with indigent patients presenting

themselves for treatment at the private institute in Jerusalem had to be defrayed by the Government at very considerable cost, while again, the Military Authorities were involved in much inconvenience and expenditure by having to send all bitten soldiers to Egypt.

It will be appreciated, therefore, that the Department's decision was based on strictly utilitarian and economic grounds.

II. SELECTION OF VACCINE

As the result of a lengthy and comprehensive survey of the various recognized modes of anti-rabic treatment, we finally resolved that the vaccine most suitable for Palestine was undoubtedly that originally introduced by Fermi of Sassari, modified by Semple, and elaborated by Harvey and McKendrick at the Central Institute, Kasauli.

The valuable reports issued by the Kasauli Institute, along with the published memoirs of Semple, on the one hand, and the close study, on the other, of the economic conditions of Palestine determined our selection.

The relative values of the three chief methods of treatment—all of which had received fair and full trial at Kasauli—are clearly enunciated by Harvey and Acton in their critical 'Examination into the degree of efficacy of anti-rabic treatment,' while the reasons which led up to the final adoption by that Institute of a carbolized vaccine in preference to the living viruses of Pasteur and Hoegyes are stated in convincing fashion.

After the inception of the Institute in 1900 the dried cord method of Pasteur was employed for seven years. While, admittedly, this treatment was highly successful, yet the occurrence, though rare, of 'accidents paralytiques' both during and after treatment could not be regarded otherwise than with disfavour. The possibility, however remote, of such accidents—believed by Harvey and McKendrick to be probably of an anaphylactic nature and due to the unavoidable introduction by this method of an excess of nerve protein during the course of injections—was responsible for a change being made to the dilution method of Hoegyes in 1907. This alteration in policy was in every way justified and the results of its adoption afforded striking confirmation of the theory advanced

by Harvey and McKendrick, for with the use of a comparatively small amount of foreign nerve protein coincided the complete cessation of cases of paralysis.

A further change of policy, however, took place in 1912 when, as a result of brilliant researches by Fermi, Semple and others, it was conclusively proved that the employment of a dead vaccine afforded a protection at least equal to that given by the living viruses of the previous methods.

Since that time the accumulated evidence of twelve years' experience has upheld beyond all question the opinion first put forward by Fermi as to the value of treatment by a carbolized vaccine.

Again, the stimulating example set by Col. Hamerton who, faced with an exceedingly difficult problem in Irak, most successfully coped with the situation there, proved a great encouragement to us, and so it was with every hope of similar success in Palestine that we established in Jerusalem, on May 1st, 1923, the Central Anti-Rabic Institute. In order, however, that the ' greatest good of the greatest number ' of inhabitants likely to be at risk might be served, ten subsidiary treatment centres in the districts, supplied with the vaccine prepared at the Central Institute, were opened on the same day.

III. PROCEDURE

It will be immediately obvious that if decentralization is to be successful, medical officers in charge of subsidiary centres must be completely conversant with all matters relating to anti-rabic procedure.

Each officer appointed to carry out such work here reports first to the Central Institute where he undergoes a full course of instruction.

During this course he has to give evidence that he has familiarized himself with the various considerations governing the policy adopted, with the whole subject of rabies and its prevention, and with every detail of procedure touching on the preparation, distribution, storage, and administration of the vaccine.

To ensure that each centre is conducted in strict accordance with instructions laid down, one or other of us carries out routine inspections.

A. General Considerations.

(1) *Was the biting animal rabid?*

An animal should be considered rabid

(*a*) if it dies from an undiagnosed disease
(*b*) if it has been killed.
(*c*) if it has disappeared

} within ten days of its biting the patient.

(*d*) if it shows marked alteration in behaviour; if, for example, it makes unprovoked assault on human beings or other animals, especially if such results in many persons or animals being bitten.

(*e*) if a jackal makes an unprovoked attack on a human being.

(2) *Has the patient been exposed to the risk of infection?*

(*a*) A person cannot be infected by (the saliva of) an animal except when that animal is actually suffering from rabies and during the ten days immediately prior to that animal's developing symptoms (two to five days before the appearance of symptoms, Roux and Nocard, Nicholas).

(*b*) The contagion, no matter what its virulence or concentration, cannot penetrate uninjured skin or mucous membrane.

In this connection, wounds which have been granulating twenty-four hours or more are considered impervious to the virus.

A very definite risk of infection is run when blood or serum oozes from cut or abrasion.

B. Procedure to be followed.

(1) *In respect of the biting animal.*

(*a*) On no account should the animal be destroyed (there are, of course, necessary exceptions to this dictum). It should be kept under observation in close confinement, when it will, if infected and infective, have developed symptoms within ten days.

If, on the other hand, the animal remains healthy at the end of ten days, it can be regarded as having been free from the possibility of causing infection at the time of biting.

(*b*) If the animal dies during the period of observation (ten days) without showing the classical signs of rabies, it should, nevertheless, be considered as having been rabid in so far as the treatment of bitten persons is concerned.

(2) *In respect of the bitten person.*

(*a*) After giving suitable local treatment to the wound (*vide* below), one must pronounce upon the biting animal. If the animal appears to be normal it should ordinarily be kept under observation for ten days, at the end of which period, if it still shows no signs of disease, it can be considered to have been non-infective and the bitten person therefore free from risk. But treatment should be begun immediately (and to this there must be no exception made), in the following circumstances :—

(*a*) When the animal develops symptoms of ill-health, dies, is killed, or escapes, during its ten days' observation period.

(*b*) When the patient has been bitten on the face, on the hand, or very severely (even through clothing) in other parts of the body.

(*c*) If the patient has been bitten by an unknown animal in the dark or while asleep in the fields (common incident enough during the summer among the *Fellaheen*).

It is, however, of first importance to note that when an animal dies during its observation period, vaccine treatment must be begun at once, and on no account whatsoever should the result of the laboratory examination of the dead animal's brain be awaited. Apart from the loss of valuable time incurred by such a wait, it must be remembered that failure to find Negri-bodies by the microscopist does not always signify that the animal was free from rabies.

(3) *In respect of the bitten animal.*

See Appendix II, paragraphs 5 and 6.

C. Diagnosis of Canine Rabies.

(1) *Clinical.*

There exists at present no better description than that given by Mohler and Eichorn in their translation of Hutyra and Marek's textbook on 'Spezielle Pathologie und Therapie der Haustiere,' published by Ballière, Tindall and Cox, and to this the reader is referred.

(2) *Laboratory.*

The brain of a dog or other animal which has died of suspected rabies or which has been shot or otherwise killed as a result of its

having bitten persons or other animals should be extracted and forwarded to the Central Institute (here part of the Central Laboratories). A simple method of extraction is to saw the head sagittally and remove the two halves of the brain intact.

When the brain has to be sent from a distance for laboratory examination, the two halves, so extracted, are placed in a wide-mouthed can and packed about with well-powdered salt. The salt must completely fill the receptacle. The lid is now to be replaced and soldered by a tinsmith prior to despatch.

We advocate this procedure of forwarding in salt in preference to that usually recommended, viz.: in separate tins containing glycerine and alcohol respectively, because the brain thus arriving in a fresh state allows of full examination.

The brain is now freed from salt by washing in sterile normal saline solution.

Laboratory investigation consists of the search for Negri-bodies and of the performance of the biological test by rabbit inoculation.

For the detection of Negri-bodies, preliminary smears from the hippocampus major or cerebellum are made and coloured with Giemsa's stain. The results are controlled by examination of sections of these parts of the brain fixed in Zenker's fluid and coloured with haematoxylin-fuchsin or Mann's stain. With the latter we have found difficulty in obtaining uniformity of results, but with haematoxylin-fuchsin we have been very successful in accordance with the following procedure :—

(*a*) Stain with haematoxylin for seven to ten minutes.

(*b*) Blue well in tap water.

(*c*) Counterstain with acid fuchsin for three minutes.

(*d*) Differentiate in tap water.

(*e*) Pass rapidly through alcohol, clarify with oil of cloves, and mount in balsam.

In regard to the Biological Test: it is performed by injecting an anaesthetized rabbit sub-durally with 0.2 c.c. of a 1 per cent. emulsion previously prepared from the medulla of a suspected animal.

The emulsion is made by rubbing-up in sterile normal saline solution a small piece of the medulla and thereafter filtering the suspension through gauze. It is then introduced sub-durally by

means of a hypodermic syringe (the needle of which is bent at a right-angle) into an opening made by a small (Eyre's intracranial) trephine in one of the parietal bones of the rabbit. It need hardly be pointed out that the strictest aseptic precautions must be observed throughout the operation. After a variable length of time in positive tests symptoms of paresis occur, and death takes place usually in from fifteen to twenty-five days.

D. Treatment.

When it has been decided that a patient requires treatment certain curative methods are employed.

(1) *Local treatment.*

Every endeavour must be made to get rid of as much of the virus deposited in the wound as possible and that without delay. Such common expedients as ligaturing, when possible, the affected part, encouraging bleeding, and freely opening up and thoroughly cleansing the wound are to be resorted to.

Cauterization of every part of the wound must now be performed with such caustics as pure carbolic or fuming nitric acid. Here we employ fuming nitric acid and consider it likely to be effective only if applied within half-an-hour of the time of biting. (Adherence to this time limit enables us to state that in our series of cases, of 1,920 persons treated, only 40 were efficiently cauterized.)

(2) *General treatment.*

The patient must, during treatment and for ten days thereafter, follow a quiet well-ordered existence. Chill, fatigue, and excitement must be avoided, while alcohol should be especially restricted.

(3) *Specific treatment* (vaccine treatment).

E. The Vaccine.

(1) *Nature and Preparation.*

The vaccine prepared by the Central Institute consists of a 2 per cent. suspension of the brain of a rabbit killed with fixed virus, in a solution of 1 per cent. phenol in distilled water.

Before being bottled and issued to the ten treatment centres, however, it is diluted with an equal quantity of normal saline

solution. Rabbits of about 1,400 grammes are inoculated sub-durally with fixed virus emulsion, and this operation is performed only on such numbers of rabbits as will presumably supply all demands for vaccine and will keep the ' strain ' going.

Attention to the technique of the trephining and inoculating operations results in a minimal number of rabbits being required, and the practically 100 per cent. success obtained here is due to the operators' observing various essentials :

(*a*) Absolute asepsis throughout.

(*b*) Rapidity in carrying out the trephine portion of the operation.

(*c*) Slowness in injecting the emulsion of fixed virus along the needle, held parallel to the dura.

(*d*) Covering the trephine opening with a pad of sterile cotton wool and maintaining slight pressure during the process of inoculation and during the slow drawing-out of the needle on completion of the operation. This procedure precludes regurgitation of the virus emulsion.

(*e*) Care in the choice of the fixed virus used for passage.

The virus in use here was originally obtained from Cairo and produces symptoms usually on the fifth, though occasionally on the sixth day after sub-dural inoculation. Whereas, for the production of vaccine, the brain of any animal showing symptoms on the fifth or sixth day is used, here ' passage virus ' is invariably selected from the brains of those rabbits that have developed symptoms on the fifth day.

This we find most important in keeping up the fixity of the virus and in obtaining uniform results of virulence.

On the eighth or ninth day, then, after inoculation, the rabbits, now moribund, are killed by chloroform, dipped in a weak solution of cresol, and dissected.

The brain, after naked-eye inspection and after cultures have been taken from it to ensure sterility, is ' extracted ' and weighed in an accurate balance. Brains showing excessive haemorrhage or other abnormality are discarded. The brain is now pounded in a sterile mortar and during this process the carbolic solution is added little by little. On an average this operation should take from fifteen to twenty minutes to complete, and when the brain has been well-emulsified into a thick sticky paste, the remainder of the

carbolic solution is slowly mixed in, until a suspension of 1 in 50 of brain substance has been obtained.

We now have a 2 per cent. brain emulsion in a 1 per cent. carbolic solution and this is filtered through two la ers of gauze to get rid of the connective and vascular tissues, and then placed in an incubator at 37° C. for twenty-four hours.

Although all workers whose methods we have studied use a carbolic solution made up with normal saline, we, after repeated observation, have preferred to employ distilled water, as this we find gives a better suspension, while precipitation of the brain matter does not occur so readily as with saline.

After the emulsion has been in the incubator for twenty-four hours, it is taken out and mixed with an equal volume of normal saline so that the vaccine now consists of a 1 per cent. brain emulsion in 0.5 per cent. carbolic solution. After passing aerobic and anaerobic cultivation tests, the vaccine is run into sterile bottles of 30 c.c. capacity in which it is distributed. Of this vaccine each person, irrespective of age, sex, or severity of bite, receives intracutaneously 5 c.c. daily, 2.5 c.c. on each side of the abdomen.

In the first year of work here, we diluted the above suspension further with an equal quantity of normal saline just before its administration, thus following the exact procedure (apart from the substitution of distilled water) described by Harvey and McKendrick and Col. Hamerton, and giving a 0.5 per cent. suspension of brain substance in 0.25 per cent. carbolic solution. In our second year, for considerations elsewhere discussed, it was decided to inject directly, without previous dilution, a 1 per cent. emulsion—and this has been in practice here since May, 1924.

(2) *Distribution, and instructions for use.*

Before despatch from the Central Institute the vaccine bottles are properly capped, paraffined, and labelled. On each label is given the following information: nature of the contents of the bottle; the dosage; the mode of administration; and directions relating to storage. In addition to this, by means of a rubber stamp are affixed further particulars:—viz., the serial number of the vaccine, the date of manufacture, and the date on which the vaccine bottle—irrespective as to whether its contents have been used or

not—is to be returned as 'out of date' to the Central Institute. This date of return is invariably three months after the date of manufacture shown on the label of each bottle.

The medical officers in charge of the various treatment centres make known their requirements fortnightly, and these indents, when met, maintain the stock held by each centre at a constant level.

The vaccine is administered in accordance with the printed instructions wrapped round each bottle :—

(*a*) Sterilize 5 c.c. Record syringe.

(*b*) Shake the bottle well.

(*c*) Withdraw 5 c.c. of vaccine by pushing the needle of the syringe through the rubber cap (previously sterilized by dipping in alcohol).

(*d*) Inject 2.5 c.c. of the vaccine on one side of the abdomen and 2.5 c.c. on the other, by inserting the point of the needle at an acute angle between the superficial and deep layers of the skin (intracutaneously).

(*e*) The complete course of treatment consists of fourteen such injections of 5 c.c. on successive days.

(*f*) The same dose is given to children as to adults, and bites of all severities are treated alike.

F. Advantages of adopting this Method of Treatment.

(1) The employment of this carbolized anti-rabic vaccine precludes the possibility of its producing *per se* the disease its object is to prevent.

Cases recorded in literature show that the injection of living or merely somewhat attenuated virus is not entirely free from danger. Babes in this connection states : 'It is very probable that fixed virus in certain cases can produce hydrophobia after subcutaneous injections. The employment of this virus, therefore, must be prohibited in the treatment of human subjects unless the organism has been first prepared with a virus sufficiently attenuated.'

Surprising, indeed, is the number of persons requiring assurance that treatment itself involves no risk, and here one is frequently asked whether, should events prove that treatment was unnecessary, any harm can possibly ensue from the administration of vaccine.

With the use of carbolized vaccine, we are fortunately able to reassure patients completely in these respects.

(2) Its use is not followed by any harmful effects.

Occasionally local hyper-reaction occurs, due to individual idiosyncrasy. Here there has been no sign of abscess formation although over 60,000 intracutaneous inoculations have been made. Further, the vaccine contains the smallest amount of nervous tissue commensurate with efficient treatment, and thereby are avoided the so-called 'post-treatment paralyses' which occasionally follow certain other methods of treatment.

(3) *Accuracy of dosage.* That uniformly successful results have been established can be claimed for no method unless dosage of vaccine can be accurately determined.

In regard to cord methods two factors must be taken into account, which militate against an accurate estimation of the number of immunizing units injected into a patient.

(*a*) Cords differ much in size, varying largely in proportion to the size of rabbit employed. A very appreciable difference must exist between the numbers of immunizing units contained in 1 c.c. of thick and in 1 c.c. of thin cord, respectively.

(*b*) Where the dried cord method is used, it will be obvious that a ten days' dried cord, from a large rabbit, will contain, normally, more living virus than a ten days' cord from a small one because desiccation proceeds more rapidly in the latter. Such difficulties do not obtain with carbolized vaccine. If care be taken to pound the brain, during manufacture, for a fixed period, say twenty minutes, and to filter the resultant suspension through gauze of uniform thickness, emulsions of unvarying strength are produced.

(4) Its dosage over fourteen consecutive days (the complete period of treatment) remains constant for all bitten persons, irrespective of age, sex, severity of bite, location and multiplicity of wounding, interposition of clothing, and different conditions requiring consideration when other methods of treatment are employed.

The reason for the application of a universal dosage lies in the fact that each case for which treatment is prescribed is regarded as being sufficiently serious to warrant the full and intensive dosage given by this method.

(5) *Economy and rapidity of production.* The use of carbolized vaccine permits of great economy both in respect of animals and of time. From a rabbit of average weight (1,400 grammes) can be produced vaccine sufficient for twelve complete courses of treatment. Further, one does not require to inoculate rabbits daily, but only as occasion demands and just often enough to maintain the ' strain.'

The actual material cost of treatment of nearly 2,000 bitten persons, here, during the past two years, has been little more than the purchase price of 150 rabbits. The time taken to produce the vaccine is short and we have clearly shown that any well-equipped laboratory can, in addition to its routine work, undertake the successful manufacture of the vaccine without additional expert staff.

An illustration of the rapidity of production is afforded by a recent occurrence when, on a farm over 100 miles from the Central Institute, 75 valuable animals were bitten by a rabid dog.

The issue of a quantity of vaccine (5,250 c.c.) sufficient for the treatment of these 75 animals was made without delay, and within nine days our reserve stock was at its normal level.

(6) Carbolized vaccine retains its maximal potency and powers of immunization for a period of at least three months if preserved under requisite conditions—away from light and in an ice-box.

It is, consequently, suitable for use in small countries where rabies is present, but too rare to justify the expenditure involved in the creation of Anti-Rabic Institutes. Such countries can purchase, from time to time, quantities of vaccine sufficient for estimated maximal requirements and their stock can be renewed quarterly at small expense. Transjordania—a country adjoining and obtaining its vaccine supplies from Palestine—affords an excellent example.

On the other hand, when living virus is employed for treatment, vaccine cannot be sent to a distance without a diminution of efficacy and without risk of its becoming infected. It is for this reason that, in our belief, carbolized vaccine is the only one of practical value in the prophylactic and curative treatment of animals.

(7) The vaccine is manufactured in a Central Institute and can be issued therefrom to any number of treatment centres where its administration to bitten persons forms part of the routine duties of the Government medical officers there. The advantages to the bitten persons of this are as follows :—

(*a*) Treatment can be begun without loss of time—an incalculable advantage in a condition where the importance of the time factor is paramount.

(*b*) The patient can be treated at or near his own home, and thereby is avoided the necessity for his undertaking long fatiguing journeys.

(*c*) The bitten person may move from town to town in accordance with the dictates of his business and be assured of an uninterrupted course of treatment.

In this connection, and to demonstrate the exceptional applicability of this form of treatment to unexpected circumstances, we would refer to an episode which occurred during 1923. In a military camp at Sarafand, two British and five Indian soldiers were severely bitten by a jackal. The jackal was shot, its brain extracted and forwarded to the Central Institute, where the finding of Negri-bodies and a positive biological test proved the animal to have been rabid at the time of biting. The bitten persons were treated at Ramleh Anti-Rabic Centre for seven consecutive days, at the end of which time the Indian regiment was ordered to proceed abroad. The Regimental Medical Officer was supplied with vaccine sufficient for seven further injections to each patient, and the remaining half of the course was administered on board ship. Reports forwarded later showed that treatment had been successful in each case.

Probably one of the most satisfactory results of our procedure, however, is that no case of hydrophobia has occurred during the past year for lack of facilities for treatment. In marked contrast to this state of affairs were the conditions (summarised in Table I) obtaining in 1922—in the year previous to the adoption of the present system of decentralisation of treatment.

(8) *Efficacy.* A.—*Theoretical considerations.* It has to be admitted, however, that the only factor determining ideal treatment is its ' Efficacy.'

If a treatment is to be universally successful it has to be able not only to prevent the onset of hydrophobia in the case of average severity, but also to ward off the disease in grave cases of short incubation periods.

The greatest advantage claimed for carbolized vaccine and its

period of administration is that it is an attempt to deal with such cases as may have short incubation periods. There can be no possibility of cure once the virus has reached the brain ; now, in a certain percentage of cases the virus does actually attach itself to the brain in or in about fifteen days ; it is obvious, therefore, that any treatment calculated to prevent the disease in these cases must aim not only at producing in the system a sufficiency of antibodies, but at producing them *within fifteen days*. Under such circumstances the time limit is of first importance, and the logic of ' intensifying ' treatments of head and face bites by prolonging them beyond the minimal period sufficient to excite and promote antibody formation in as great an area as possible is by no means clear. Surely here the thing to do is to increase the dosage administered within the minimum period of time at one's disposal. The importance of realising this cannot be over-estimated, and a glance at this modified table of Bauer (II) will show short incubation periods to be by no means rare. Further, in Table I, are two such cases.

Table II (after Bauer).

Number of days incubation	Percentage of total
1–19	8·24
20–39	28·34

A short intensive course of treatment might likewise prove of extreme value in those cases where bitten persons report at an institute late. Here again time is all-important and the maximum dosage bearable should be administered in the shortest possible period.

Moreover, the use of brain matter instead of cord doubtless contributes towards the efficacy of carbolized vaccine and its superiority over cord methods.

Brain matter is said by Nitsch to be ten times more virulent

than spinal cord. In using brain, therefore, we are giving a larger proportion of specific antibody-producing substance and a smaller one of the useless, probably harmful, nervous tissue than is practised in methods of cord immunization.

B.—*Practical results*. We shall first record the experimental and then the practical evidence on which our assertions as to the efficacy of this method are based.

(1) *Experimental.*

The subjoining Tables III and IV are self-explanatory.

TABLE III

Immunizing Experiments.

No. of experiment	Animal	Duration of Treatment	Quantity of 1% carbolized vaccine injected subcutaneously	Test	Result
1	Rabbit	14 days	28 c.c.	0·2 c.c. of 1% fixed virus emulsion introduced subdurally 15 days after last injection.	Lived
2	Rabbit	14 days	28 c.c.		Lived
3	Rabbit	14 days	28 c.c.		Died in 15 days
4	Rabbit	14 days	28 c.c.		Lived.
5	Rabbit	14 days	28 c.c.		Died in 18 days
6	Rabbit	Not treated	None		Died in 8 days
7	Rabbit	Not treated	None		Died in 8 days
8	Rabbit	Not treated	None		Died on 9th day

The temporary nature of the immunity conferred is well illustrated in regard to rabbit No. 2. Without prior immunization this animal was innoculated with 0.2 c.c of 1 per cent. emulsion of fixed virus, fourteen months later, and died in eight days.

To show the evidence of immunity in the serum of rabbits treated with 1 per cent. carbolized vaccine, we have, guided by examples from existing literature, performed the experiments recorded in the next table.

TABLE IV.

Animal immunized	Vaccine used for immunizing	Time after completion of treatment when serum was tested	Proportion of serum and 1% fixed virus emulsion tested	Tests applied to mixture of serum and virus	Result
Rabbit	1% carbolized anti-rabic	15 days	Serum 1 c.c. + fixed virus 1 c.c. incubated 2 hours at 37°	Subdurally into rabbit	Remained well
Rabbit	do.	do.	do.	do.	do.
Rabbit	do.	do.	do.	do.	Died 12th day
Rabbit	do.	do.	Only fixed virus 1% emulsion	do.	Died after 8th day
Rabbit	do.	do.	do.	do.	do.
Rabbit	Not immunized	Directly	do.	do.	Died in 8 days
Rabbit	do.	do.	do.	do.	do.

(2) *Deductions from treatment of bitten persons.*

In the various records given below it has been considered advisable to include in the first part of each table, mostly for purposes of interest and comparison, the total number of bitten persons attending the various anti-rabic treatment centres; the number whose treatment was considered to have been unnecessary as reckoned by the biting animals having remained alive and well after ten days' observation, and the number of cases where a regular and complete course of treatment was carried out. On the last number alone have the death and so-called 'failure' rates been estimated.

The only fair and accurate means, however, of arriving at the actual success or failure of any line of treatment is to base the final calculations only on the number of bitten persons treated who had been definitely or presumably exposed to risk of infection.

The second part of each table, therefore, consists of an enumeration of :

(1) Those persons definitely-at-risk as proved by :—

(*a*) Laboratory investigation.

(*b*) Veterinary officer's certificate obtained after observation of the biting animal.

(2) Those persons presumably-at-risk, i.e., who had been bitten by animals fulfilling the conditions laid down under A. General Considerations, paragraph 1, items (*d*) and (*e*).

(3) Death and 'failure' rates calculated on a total of (1) + (2).

Statistics from May 1, 1923, to April 30, 1925.

Part 1.

Number of persons treated at various Government anti-rabic treatment centres in Palestine	1,920
Number of treatments interrupted or unnecessary	470
Number of regular and complete treatments administered ...	1,450
Total Deaths	12
Death rate	0·82%
Deaths occurring later than 15 days after completion of Treatment	4
'Failure' rate	0·27%

Part 2.

Number of persons definitely-at-risk—	
(*a*) Proved by laboratory investigation	270
(*b*) Proved by veterinary officers' certificates	123
Number of persons presumably-at-risk	420
Death rate	1·47%
'Failure' rate	0·49%

The above statistics, however, represent the results obtained from the combination of two definite work periods differentiated by alteration in daily dosage; thus, whereas in the period 1923-24, treatment consisted of the daily administration of 2 c.c. carbolized vaccine over fourteen consecutive days, during 1924-25 a dosage of 5 c.c. daily over the same number of days was substituted.

The results achieved during the two periods afford interesting comparison.

Part 1.	Period 1923-24 Dosage 2 c.c. of 1% emulsion	Period 1924-25 Dosage 5 c.c. of 1% emulsion
Number of persons treated	886	1,034
Unnecessary treatments	138	332
Regular and complete treatments	748	702
Total deaths	9	3
Death rate	1·2%	0·4%
'Failures'	4	0
'Failure' rate	0·5%	0
Part 2.		
Number of persons definitely-at-risk—		
(*a*) Proved by laboratory investigation	180	80
(*b*) Proved by veterinary officers' certificates	48	75
Number of persons presumably-at-risk ...	213	207
Death rate	2%	0·8%
'Failure' rate	0·9%	0

It will be observed that 307 and 340 persons belonging respectively to the 1923-24 and 1924-25 periods are not included in Part II, although they have been bitten by animals fulfilling the conditions laid down in A. General Considerations, paragraph 1, items (*a*), (*b*) and (*c*)—conditions which demand the immediate treatment of the bitten persons from the point of view that the biting animals are to be considered rabid. Such number, if included, would obviously lessen both the death and 'failure' rate, thereby elevating the degree of efficacy of this form of treatment.

It is generally admitted that a certain percentage of cases cannot be benefited by any form of anti-rabic treatment. In such cases the incubation period is very short and the virus reaches the brain before a sufficient degree of immunity can be conferred. Excellent examples are afforded by numbers 2, 4, and 6, in Table I, and by the first line statistics of Table II.

On the other hand it must be admitted that when a patient dies of hydrophobia, say one, two, or three months after completing a full and regular course of treatment, this case cannot be included in the above category but must be considered as a failure of treatment in that either the effect of treatment was nil or that it sufficed merely to delay the arrival of the virus in the brain, thereby prolonging the incubation period of the disease, which latter alternative is exemplified in Table III, number 3 and 5, of our immunizing experiments.

Now in view of the results obtained in the 1923-24 period, with the administration of 2 c.c. daily for fourteen days, it may be a matter for surprise that we resolved to make an alteration in dosage to 5 c.c. daily over a similar period.

However, a study of the following four cases—our only failures of treatment as judged by the conventional standards, viz., when hydrophobia supervenes later than fifteen days after completion of a regular course of treatment—was mainly instrumental in bringing about our decision.

Case 1. Male, aged 10 years, severely bitten on forehead, hand and leg through naked skin by a jackal, reported one day after bite for treatment, the wound not having been cauterized before arrival. Treatment was regular over 14 days. Symptoms developed 59 days after the date of bite, and 44 days after completion of treatment.

Case 2. Male, aged 35 years, severely bitten on face, eyelid, and thumb by jackal through naked skin, reported three days after bite, the wound not having been cauterized, and attended regularly for treatment. Symptoms appeared 81 days after the bite and 65 days after completion of treatment.

Case 3. Male, aged 8 years, bitten slightly on upper extremity through naked skin by a dog, arrived two days after bite with wound not cauterized. Symptoms occurred 36 days after the bite and 21 days after completion of treatment.

Case 4. Male, aged 30 years, bitten severely through clothing on lower extremity by a dog, arrived immediately after for treatment and had wound cauterized with fuming nitric acid. Treatment was administered regularly over 14 days. Hydrophobia supervened 39 days after the bite and 26 days after completion of treatment.

We felt convinced that these four cases died, not as the result of any special exaltation of a virus (virus de rue renforcé) or shortness of incubation period, but because the treatment administered had not been sufficiently intensive : with this conclusion Professor Fermi, of Sassari, to whom we submitted the facts, was in complete agreement. (It is, however, worthy of record that thirteen other persons, bitten by the animals inflicting bites on the four persons cited, received full and regular courses of vaccine also, and all thirteen are to-day alive and well, one-and-a-half years after completion of treatment.)

As a result an alteration in dosage was effected for all cases, on May 1st, 1924, after a month's preliminary trial had demonstrated beyond question the ability of children and adults alike to tolerate the daily increase from 2 to 5 c.c.

It was resolved by us to make an increase in daily dosage rather than in the period of vaccine administration, so that the method of treatment might be made as widely applicable as possible and especially with regard to such cases as might have short incubation periods.

Although we consider that results in human beings have justified the alteration, yet Table V will demonstrate our inability to adduce experimental proof of the value of increased dosage in laboratory animals.

Further, from this table it would appear that :—

(*a*) Better results in rabbits are obtained by the giving of small doses over a number of days (14) than of larger doses over a shorter period.

(*b*) It is impossible to reduce below a certain limit the time over which the total quantity of vaccine, ordinarily sufficient for complete immunization, can be usefully administered.

(*c*) As large a dose as 30 c.c. of a 0.5 per cent. emulsion in 0.25 per cent. carbolic may be given without the production of toxic symptoms.

TABLE V

Experimental animal used	Dose and Period for Immunization					Whether or not animal survived treatment	Subdural Test
	2 c.c. daily for 14 days	4 c.c. daily for 14 days	6 c.c. daily for 10 days	15 c.c. daily for 3 days	One injection of 30 c.c.		2 weeks after treatment tested with 0·2 c.c. of 1% emulsion of fixed virus
Rabbit	1	...	...	...	...	Survived	Survived
Rabbit	1	...	...	...	...	Survived	Died
Rabbit	1	...	...	...	...	Survived	Died
Rabbit	1	...	...	...	...	Survived	Survived
Rabbit	...	1	...	...	...	Survived	Survived
Rabbit	...	1	...	...	...	Survived	Died
Rabbit	...	1	...	...	...	Died	...
Rabbit	...	1	...	...	...	Survived	Died
Rabbit	...	...	1	...	...	Survived	Survived
Rabbit	...	...	1	...	...	Survived	Died
Rabbit	...	...	1	...	...	Survived	Died
Rabbit	...	...	1	...	...	Survived	Died
Rabbit	...	...	...	1	...	Survived	Died
Rabbit	...	...	...	1	...	Survived	Died
Rabbit	...	...	...	1	...	Survived	Died
Rabbit	...	...	...	1	...	Survived	Died
Rabbit	...	...	...	...	1	Survived	Died
Rabbit	...	...	...	...	1	Survived	Died
Rabbit	...	...	...	...	1	Died	...
Rabbit	...	...	...	...	1	Survived	Died

We would now draw attention to the fact that in the records of statistics published above have been included figures for total deaths and for the so-called failures of treatment as well as their respective percentages.

'Failure,' in accordance with convention, is applied only to

those cases which, in spite of treatment, develop hydrophobia later than fifteen days after a complete anti-rabic course has been administered. By the same convention are excluded from ' failures ' all bitten persons dying during treatment or within fifteen days of its completion.

(Remlinger, e.g., has shown that during 1901-1908, in Constantinople, where the original Pasteur method is utilised, of persons dying despite treatment no fewer than 80 per cent. could be so excluded.)

Statistics based on this mode of computation will obviously vary with regard to :—

(*a*) Length of time taken to adminster a course of treatment.

(*b*) Lateness of arrival of the bitten person for treatment.

The longer a person defers reporting for treatment, and the longer the period of treatment—the lower the ' failure rate ' of an institute.

It is felt that such statistics tend unduly to emphasise the value of certain forms of anti-rabic treatment and especially of such as normally require a lengthy period for adminstration.

We realise that this method of recording deaths and failures would be ideal, if all institutes were to employ the same method whereby the same dosage of a similarly prepared vaccine was administered during the same period of time. As matters stand at present, however, when anti-rabic methods are many and diverse, it is our opinion that the only way to present statistics which can admit of real comparison is a simple enumeration of the deaths occurring at an institute and the percentage such bears to the total attending population proved or believed to be at-risk.

Deaths from hydrophobia should then be reported on in full, with especial reference to the duration of treatment, to the number of days intervening between completion of treatment and onset of symptoms, to the lateness of arrival for treatment, and to the irregularity of attendances during the course.

This information, in addition to that supplied in Appendix I, will generally enable an impartial pronouncement to be made upon such cases in their relation to treatment.

G.—Records. It was at once recognised that one of the criticisms most likely to be levelled against decentralisation of treatment would be the presumed unreliability of the records of available information.

With the view, therefore, of meeting just such a charge we evolved the present procedure of keeping records of the particulars of all patients treated in the various Government Centres.

Appendix I gives the form which enables a complete register of cases undergoing anti-rabic treatment to be kept at each treatment centre by the medical officer in charge. Immediately a patient has completed his course, the original form is submitted to the office of the Central Institute for filing, while the duplicate is retained at the treatment centre concerned. Three months after the last day of completion of treatment, a final report on the patient is submitted to the Central Institute by the responsible medical officer and this report certifies to the patient being in good health (or otherwise) on that date. This procedure, we consider, allows of the ready compilation of exact statistics.

IV. SUMMARY

1. Carbolized Anti-rabic Vaccine is an efficient and safe treatment for persons bitten by rabid animals.

2. It can be manufactured without additional staff in any well-equipped laboratory.

3. It can be distributed to any number of treatment centres where as good results attend its use as at the place of its production.

4. Better results have followed the employment here of a dosage of 5 c.c. daily of a 1 per cent. emulsion, over fourteen days, than of 2 c.c. of the same emulsion over the same period.

5. Carbolized vaccine is most practicable in the curative and prophylactic treatment of farm animals.

It can be easily administered in rural districts by veterinary officers.

6. It is a great advance on older methods of treatment in the wideness of applicability and economy of production of the vaccine. It is at once the most economical and utilitarian mode of treatment.

7. Bitten persons can be treated at or near their own homes, and thus—the all-important consideration of immediate treatment aside—they are spared the expenses connected with travel to, and residence in, a strange town.

ACKNOWLEDGMENTS

Our thanks are due to the Director of Health, Col. G. W. Heron, D.S.O., O.B.E., for his unfailing encouragement and for his affording us facilities which rendered the whole scheme of decentralisation possible. We are indebted also to Dr. Miftah, Director of the Pasteur Institute, Cairo, for many favours, but especially for his ungrudging assistance in the training of our personnel.

For the sake of completeness, we have considered it advisable to add three appendices :—

I. The form employed to register cases undergoing anti-rabic treatment.

II. Regulations for the control of rabies (in which is incorporated the procedure to be adopted in the case of the bitten animal). These regulations made under Art. 43 of the Ottoman Law concerning Diseases of Animals, and drawn up by Col. E. R. Sawer, Director of Agriculture, have been in force since December, 1924.

III. Further measures adopted to free Palestine from rabies and hydrophobia.

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APPENDIX I

Local Form O. M. 186

Department of Health

REGISTER OF CASES UNDERGOING ANTI-RABIC TREATMENT.

No. Date District

Information about patient :

Name Age Profession
Residence and Address
Nationality Sent by
Date of bite Animal inflicting bite
Station where bitten

Wounds

	Number	Gravity	Bitten on naked skin or through Clothing
Head and neck			
Upper extremity			
Lower extremity			
Body			

Have wounds been previously treated ? (cauterized) and when ?

Information about the animal.

Owner of animal Address
What has become of the animal ?
Other persons bitten, with names and addresses
Other information (e.g. " dog bit unprovoked ")

Diagnosis.

1. Condition of animal from enquiry
2. Microscopical Researches (Negri bodies)
3. Experimental inoculation ? Result Date

Treatment.

1. When started 2. Vaccine and Dosage 3. Serial No. of Vaccine

4. Attendances

Month															
Dates															

5. Conduct of patient during treatment
6. Accidents, if any, during treatment
7. Other remarks

Signature of M. O.

Final Report on Patient.

No.

On being three months after the last day of the completion of treatment, the patient is alive in good health (or otherwise) and is living at (address)

District Signature of M. O.

Date

ACKNOWLEDGMENTS

Our thanks are due to the Director of Health, Col. G. W. Heron, D.S.O., O.B.E., for his unfailing encouragement and for his affording us facilities which rendered the whole scheme of decentralisation possible. We are indebted also to Dr. Miftah, Director of the Pasteur Institute, Cairo, for many favours, but especially for his ungrudging assistance in the training of our personnel.

For the sake of completeness, we have considered it advisable to add three appendices :—

I. The form employed to register cases undergoing anti-rabic treatment.

II. Regulations for the control of rabies (in which is incorporated the procedure to be adopted in the case of the bitten animal). These regulations made under Art. 43 of the Ottoman Law concerning Diseases of Animals, and drawn up by Col. E. R. Sawer, Director of Agriculture, have been in force since December, 1924.

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4. Attendances

Month															
Dates															

5. Conduct of patient during treatment
6. Accidents, if any, during treatment
7. Other remarks

Signature of M. O.

Final Report on Patient.

No.

On being three months after the last day of the completion of treatment, the patient is alive in good health (or otherwise) and is living at (address)
District Signature of M. O.
Date

APPENDIX II

REGULATIONS FOR THE CONTROL OF RABIES.

1. Every person having had in his possession or under his charge an animal affected with or suspected of Rabies shall give notice of the fact with all practicable speed to the Mukhtar, President of the Municipality or Police as the case may be. Failure to give such notice renders the person liable to a fine of £1 to £5 or to imprisonment not exceeding one month.

2. It is the duty of Mukhtars, Presidents of Municipalities and Police, on receiving such notice, to destroy the affected animal or to place it in strict isolation, and to transmit the information immediately to the District Governor or District Officer who will delegate the Veterinary Inspector to institute inquiries.

3. On confirmation of the disease and receipt of the report of the Veterinary Inspector, the District Governor or District Officer shall form a sanitary commission in accordance with the provisions of the said Ottoman Law, to execute the measures necessary for the control and suppression of the disease.

The commission shall be composed of the District Officer as president, and the Veterinary Officer, a representative of the Public Health Department, the Local Commandant of Police, and a member of the Municipal Council as members.

4. Every animal affected with Rabies shall be destroyed. Any animal whose behaviour leaves no doubt as to its being rabid shall be destroyed on the spot, and its body, in the case of dogs, cats and small animals, taken to the nearest District Veterinary Officer for disposal.

5. Animals bitten by rabid animals shall be dealt with as follows :—

(*a*) Donkeys, dogs, cats, monkeys, etc., shall be destroyed.

(*b*) Local camels ; bulls, cows, calves, oxen, sheep and goats shall be slaughtered ; but provided such animals are slaughtered within seven days of the date when first bitten, their carcases, if free from other diseases, may be exposed for sale as food.

(*c*) Valuable horses, mules, bulls, cows and calves shall be destroyed, or (1) strictly isolated for four months under the observation of Government Veterinary Officers and on premises approved by the Department of Agriculture, and (2) vaccinated with Anti-rabic Vaccine at the owner's risk and cost.

It is prohibited to sell such animals for any purposes whatsoever, during the period of observation.

6. Every animal bitten by a suspectedly rabid animal, and any dog which has been in contact with a suspectedly rabid dog shall either be destroyed or shall be strictly isolated :—

(*a*) Under the observation of Government Veterinary Officers, and

(*b*) In special cages, kennels, or stables, or on premises approved by the Department of Agriculture ; and

(*c*) At the entire risk and expense of the owner ; and

(*d*) For a period of six months in the case of dogs, or four months in the case of herbivorous animals or in both cases for such a period as will allow the diagnosis to be confirmed by the District Veterinary Inspector.

7. Every animal which has bitten a human being shall be placed in strict isolation and under observation for a period of at least ten days at the owner's risk and expense.

8. In no case may such animals be detained for purposes of observation by any private person or institution.

9. Animals will be destroyed by order of the District Officer or the District Veterinary Inspector, and no compensation will be paid in respect of such animals when they are :—

(*a*) Rabid or suspectedly rabid, or bitten by such animals ;

(*b*) In contact with a rabid or suspectedly rabid dog or other carnivorous animals.

10. The carcases of rabid or suspectedly rabid animals will be burned or deeply buried unskinned, but only after the examination by a District Veterinary Inspector or on the authority of the District Officer, in places selected by them.

11. The District Governor or President of a Sanitary Commission formed under the Law, shall in any locality where a case of rabies has occurred, issue a notice proclaiming the measures to be taken to control and suppress the disease, and the owners or persons in charge of animals shall observe and comply with such regulations.

12. The Administrative Authority, after notifying the public in the town or area, may proceed at any time to poison or destroy in any manner vagrant, stray, ownerless or collarless dogs and dogs not carrying the municipal tally.

13. Any person who fails to comply with any of the foregoing regulations or orders issued by the District Governor or Sanitary Commission for the Suppression of Rabies, or who does not assist in execution of such orders, shall be liable to prosecution before a magistrate under Art. 39 of the Ottoman Diseases of Animals Law of the 5th December, 1910 (1329 Moslem year), and on conviction to imprisonment not exceeding three months or to a fine not exceeding £20.

APPENDIX III

Apart from the preparation of a suitable vaccine for the treatment of persons bitten by rabid animals further measures were adopted to deal with the menace.

(*a*) The extermination of jackals and stray dogs—the common transmitters of the disease to human beings ;

(*b*) The education of the public in town, district and village regarding the nature of the disease, its method of transmission and the action to be taken by an individual who has been exposed to risk of infection from being bitten by a dog or other animal.

In regard to (*a*), the following action was taken by the Departments of Agriculture, Police and Prisons, and Health acting together :—

(1) The organisation of a constant campaign conducted by the Gendarmerie in country districts to lay bait poisoned with strychnine capsules in places frequented by jackals and pariah dogs. The campaign is carried out

along lines carefully worked out by the Department of Agriculture and during the present year has resulted in the finding of over 1,000 dogs and 750 jackals. The number of animals killed is known to exceed those actually found dead, as many which have swallowed poisoned baits travel some distance before the poison takes effect ;

(2) In town areas the first step towards reducing the numbers of ownerless and pariah dogs was to regulate the registration and licensing of dogs kept by householders as guards or domestic pets. This registration is effected by the staff of the Municipalities under model regulations drafted by the government Departments concerned and adopted by all towns. Municipal employees are authorised to apprehend and destroy all dogs found at large not bearing on their collars a numbered tally indicating that they have been duly licensed.

When the number of ownerless dogs is found to be increasing in any given area of a town in spite of these measures, the assistance of the Police is called upon and the animals are destroyed by shooting.

From 1st January, 1923, until 30th June, 1925, over 20,000 ownerless dogs were destroyed.

Municipalities furthermore are required to make arrangements for the safe custody of dogs whose observation by a veterinary officer is necessary on account of their suspicious behaviour or unwarranted attacks on human beings. This is effected by the Municipalities providing 'Observation Kennels' constructed in accordance with plans prepared by the Department of Health and approved by the Chief Veterinary Officer.

In regard to (*b*), articles have been written for publication in the newspapers of the country drawing attention to the dangers of hydrophobia and informing the pub'ic where to present themselves for anti-rabic treatment if they chance to have been bitten by a dog or other animal. Pamphlets have been written in all the official languages for distribution in schools. Through the medium of the Department of Education these pamphlets reach a very large number of homes throughout the country and opportunity is taken by the teachers when giving these papers to the children to explain to them in short and simple language the essential facts concerning the disease and its prevention.

This educative work is of great value, for in hydrophobia no less than in other communicable disease the intelligent co-operation of the public is essential to secure the success of preventive measures undertaken by the Government.

ERYTHROCYTOSIS IN ARTIFICIALLY-INOCULATED MALARIA

BY

G. de M. RUDOLF

AND

J. C. RAMSAY

(*Claybury Mental Hospital*)

(*Received for publication* 12 *September*, 1925)

In eleven cases of general paralysis inoculated subcutaneously with benign tertian malarial blood, Pijper and Russell (1925) found that an erythrocytosis occurred before the anaemia developed. In the graph showing the mean of the daily observations on these cases, the increase of the red cells occurs before the onset of the fever and is to the extent of about 750,000 cells per c.mm. In the charts of two cases, however, the erythrocytosis is shown to persist until the eleventh and twelfth days of the fever. The greatest increase recorded by these observers was 2,000,000 cells per c.mm.

On the other hand, R. M. Gordon (1925) found no increase of the red cells in three general paralytics inoculated with benign tertian malaria by means of anopheline mosquitos. Ben-Harel (1923) found in a series of 23 blood inoculations of *Proteosoma praecox*, in canaries, that the red cells diminished in numbers before the parasites were found in the blood stream.

The observations described in this paper were made upon cases of general paralysis inoculated with benign tertian malaria at Claybury Mental Hospital. Case No. 1 was inoculated by means of anopheline mosquitos by Lieut.-Col. S. P. James. The other cases were inoculated with defibrinated malarial blood by means of the method described by one of us elsewhere (1925). Cases Nos. 1 and 2 were females, 62 and 42 years of age ; cases Nos. 3 and 4 were males, 40 and 50 years of age.

The red cell estimations were made with the Thoma-Zeiss counting apparatus and the haemoglobin estimations by means of Oliver's haemoglobinometer. The blood was collected between 9.30 a.m. and 10 a.m. every day from cases Nos. 1 and 2, and at 5.30 p.m. from

cases Nos. 3 and 4. Three red cell counts were performed in cases Nos. 3 and 4, the averages being taken as the correct readings. In cases Nos. 1 and 2 the red cell count was invariably repeated several times if there was much variation from the previous day's count. The average was taken of the two counts which approached each other most nearly. These methods of counting the red cells were used on account of the great error there is in the usual red cell count, Gordon (1925) placing it at 500,000 cells per c.mm. The temperature of each patient was recorded every four hours unless it was above normal; it was then recorded each hour until normal was regained. No drugs, other than those mentioned on the charts, were given.

Red Cells. The results obtained from the red cell counts are shown on the charts. All four cases show very clearly an erythrocytosis preceding the anaemia. The relation of this increase of the red cells to the onset of the fever was very variable. In case No. 1 it occurred before and during the onset, in case No. 2 it coincided with the onset, in case No. 3 the greatest increase occurred after the fifth rise of temperature and in case No. 4 after the eighth. As the count was not commenced until six days after the onset of the fever in this case, it is possible that there had been an erythrocytosis previous to the one recorded.

This erythrocytosis is in agreement with the findings of Pijper and Russell, but not with those of R. M. Gordon or Ben-Harel. As neither of the two latter workers record observations made every day, it is possible that an erythrocytosis occurred between the observations. Cases Nos. 1 and 2 of the present series show that the erythrocytosis may persist for only a few days. Gordon does, however, describe one case on whom red cell counts were performed every day, but the estimations were not commenced until the ninth day of infection. There were then 6,000,000 red cells per c.mm. As this is a comparatively high figure for an untreated general paralytic in England, perhaps the count represents an erythrocytosis. In case No. 1 of the present series, 6,000,000 red cells per c.mm. were found on the tenth day of infection.

The duration of the anaemia is of interest. In cases Nos. 1 and 3 it persisted for at least 11 and 13 days after the commencement of the quinine. In case No. 4 it persisted for at least six days after the course of quinine had been started, becoming more

profound although no further febrile paroxysms had occurred. This case corresponds with one described by Gordon. In this patient, a female aged 13 years, the red cells continued to fall although the parasites had disappeared from the peripheral blood. In case No. 4 of the present series the same condition occurred. The lowest red cell count found in the present series was 1,300,000 cells per c.mm.

Recovery from the anaemia took place in a comparatively short time. In cases Nos. 1, 2 and 3, the red cells reached 5,000,000 per c.mm. in about three weeks. During this period the cells increased by 1,700,000 per c.mm. in case No. 1, by 2,600,000 per c.mm. in case No. 3, and by 3,300,000 per c.mm. in case No. 2. In these cases the degree of the anaemia did not influence the time required for normal to be regained. James (1920) gives a chart, after Ziemann, showing regeneration of the red cells after naturally-acquired malaria. In this case the normal was regained 16 days after the anaemia. Case No. 4 shows a more rapid recovery, 5,000,000 red cells per c.mm. being reached in about a week after the anaemia. In cases Nos. 1 and 2, both of whom received neo-kharsivan in addition to quinine, the regeneration of the red cells was no more rapid than in case No. 3, and less rapid than in case No. 4. Neither of the two latter cases were given this preparation. As neosalvarsan has a definite parasiticidal action on *Plasmodium vivax*, both in the naturally-acquired infection (D'Esterre, 1920; Nieuwenhuyse, 1921; Johnson, Gilchrist and Hay-Michel, 1921) and in the artificially-inoculated form (Pijper and Russell, 1924), it might be expected that the parasites would be destroyed more rapidly in cases Nos. 1 and 2, than in cases Nos. 3 and 4 and, consequently, the red cells would be regenerated more rapidly. This did not occur.

In cases Nos. 3 and 4, red cell counts were continued after the normal had been regained. In each case an erythrocytosis was found. The highest count obtained in case No. 3 was 5,920,000 cells per c.mm., and in case No. 4, 6,280,000 per c.mm. This post-anaemia increase of red cells has been observed, according to Ben-Harel, in the naturally-acquired infection, and this worker noted it in canaries who had suffered from infection with *Proteosoma praecox*. In two of the birds the erythrocytosis persisted for a little over six

months. Of the two cases under review, No. 3 showed a count of 4,990,000 cells per c.mm., with a haemoglobin percentage of 65 on July 10th, nearly six months after the last rigor, while No. 4 gave a count of 5,870,000 cells per c.mm. and haemoglobin at 90 per cent. on July 22nd, exactly six months after the last rigor. Although the highest point reached in these two cases was not permanent, in neither case was the count lower than it had been before the malarial anaemia. In case No. 4 it was about one million higher.

Haemoglobin and Colour-Index. In cases Nos. 3 and 4 estimations were made of the haemoglobin. The results, with the colour-indices, are shown on the charts. The haemoglobin does not vary to the same extent as do the red cells and the colour-index remains low. The colour-index in case No. 4 is of the secondary anaemia type throughout. This corresponds with the two cases reported by Gordon. This agreement is not seen in case No. 3, for the index exceeds unity on two occasions in this patient. In both cases it falls very low at certain periods.

Number of Parasites. In cases Nos. 1 and 2 the relative number of parasites was found by counting the total number of asexual forms in 100 fields of the microscope, using 1/12th in. oil-immersion objective and thin blood-films. Although the actual number of asexual parasites was very different in the two cases, both patients show that there is a tendency for the temperature to vary with the number of parasites in each patient. The temperatures of the two cases were recorded every hour when above normal, at other periods every four hours. In the cases investigated by Pijper and Russell the temperatures were recorded every four hours throughout. In their cases there is no clear relationship between the number of parasites and the degree of fever.

Previous Malaria. Three of Pijper and Russell's nine cases were known to have had previous attacks of malaria, whilst the remainder had a doubtful history with regard to this point. Of the present series, cases Nos. 1 and 2 are known not to have suffered from the infection previously. It is clear, therefore, that the increase of the red cells preceding the anaemia does not necessarily bear any relation to previous malaria, for the increase was found both in patients who had suffered from previous malaria and in those who had not.

Sex. As the increase of the red cells preceding the anaemia was found in both female (Nos. 1 and 2) and male (Nos. 3 and 4) cases, the erythrocytosis is not dependent upon the sex of the patient.

Mode of Inoculation. Case No. 1 was inoculated by means of anopheline mosquitos, the remaining patients by means of subcutaneous injection of malarial blood. Pijper and Russell's cases were inoculated by the latter method. The pre-anaemia erythrocytosis was found in all the cases. The increase of the red cells is not dependent upon the method of inoculation, therefore, so far as these two methods are concerned.

SUMMARY

(1) In four cases of general paralysis inoculated with benign tertian malaria an erythrocytosis was found to precede the anaemia. The erythrocytosis occurred whether the inoculation was performed by means of mosquitos or by subcutaneous injection of malarial blood. It was independent of the sex of the patient and of a history of previous malaria. The succeeding anaemia occurred, or persisted for several days after the cessation of the fever. Regeneration of the red cells was complete within three weeks, although the degree of anaemia was very different in the four cases. An erythrocytosis was found to follow the anaemia.

(2) The haemoglobin was found, in two cases, to vary with the red cells, but regeneration was less rapid. The colour-index was, as a rule, of the secondary anaemia type. At certain periods it became as low as ·5.

(3) In two cases the number of parasites was found to vary approximately with the degree of fever, although the number of parasites was very different in the two cases.

Our thanks are due to Dr. G. F. Barham for permission to publish the above observations from the records of Claybury Mental Hospital, and to Dr. F. Paine for his kind assistance.

We are again indebted to Lieut.-Col. S. P. James for his kind advice.

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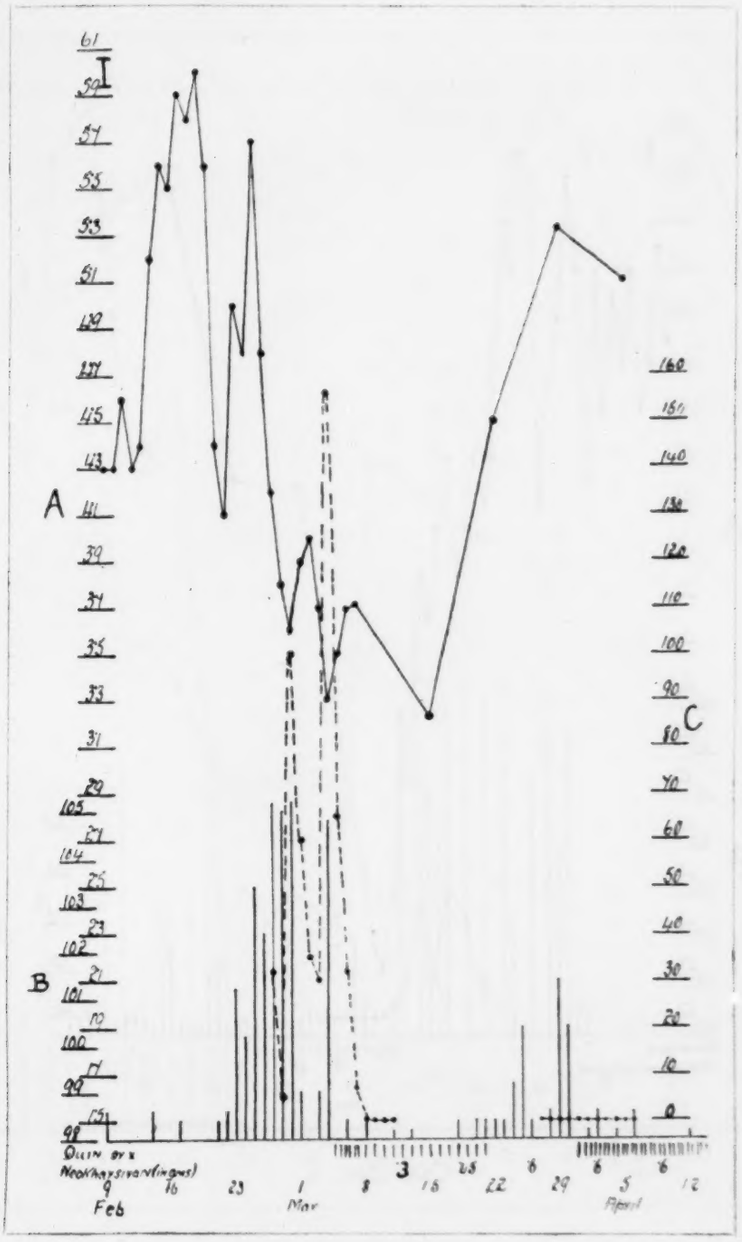
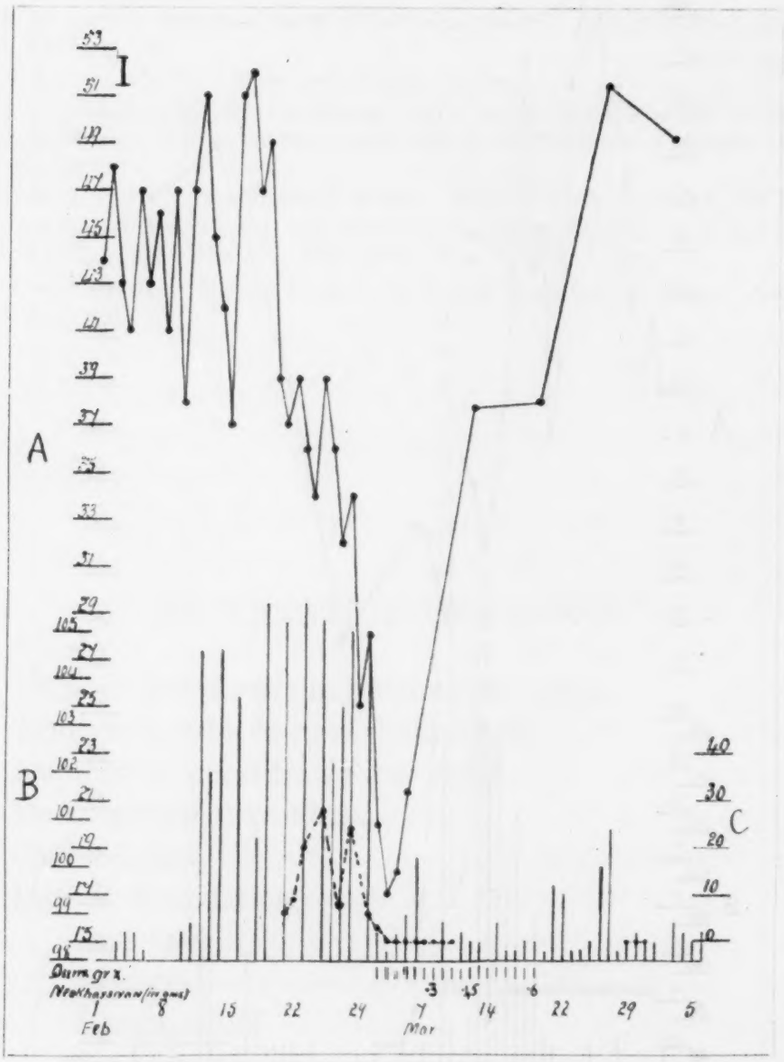
EXPLANATION OF CHARTS

A. Number of red cells in millions per c.mm.
B. Temperature in degrees, Fahrenheit.
C. Number of parasites in 100 fields.
D. Haemoglobin percentage.
E. Colour-Index.
I. Day of Inoculation.

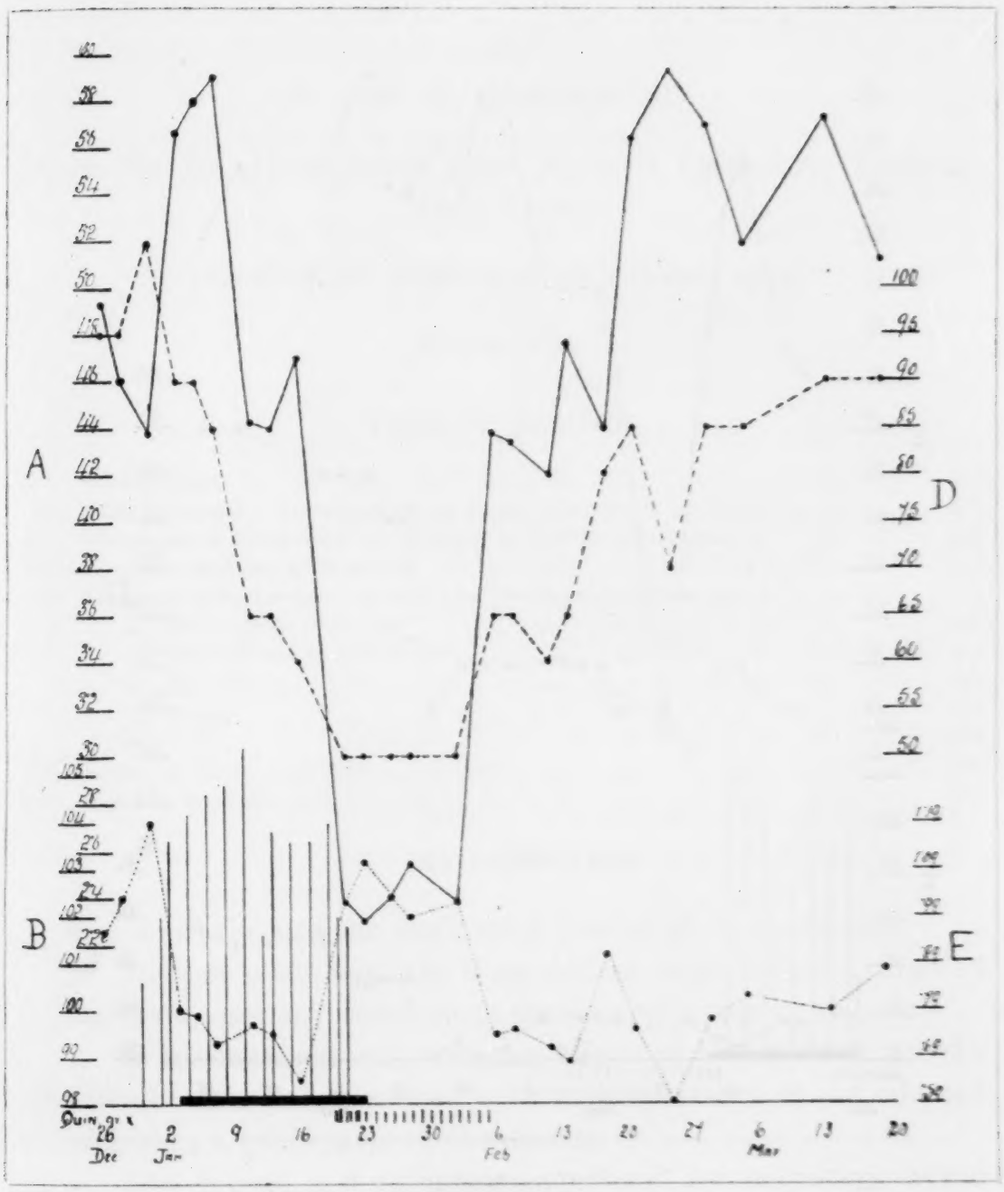
Red cells ———
Parasites – – – – – – and ▬▬▬
Haemoglobin – – – – – – –
Colour-index .....
Temperature |



CASE NO. 1


I
A
B
C
Durm gr x
Neosalvarsan (in gms)
1 Feb
8
15
22
29
7 Mar
14
22
29
5
40
30
20
10
0

Case No. 2



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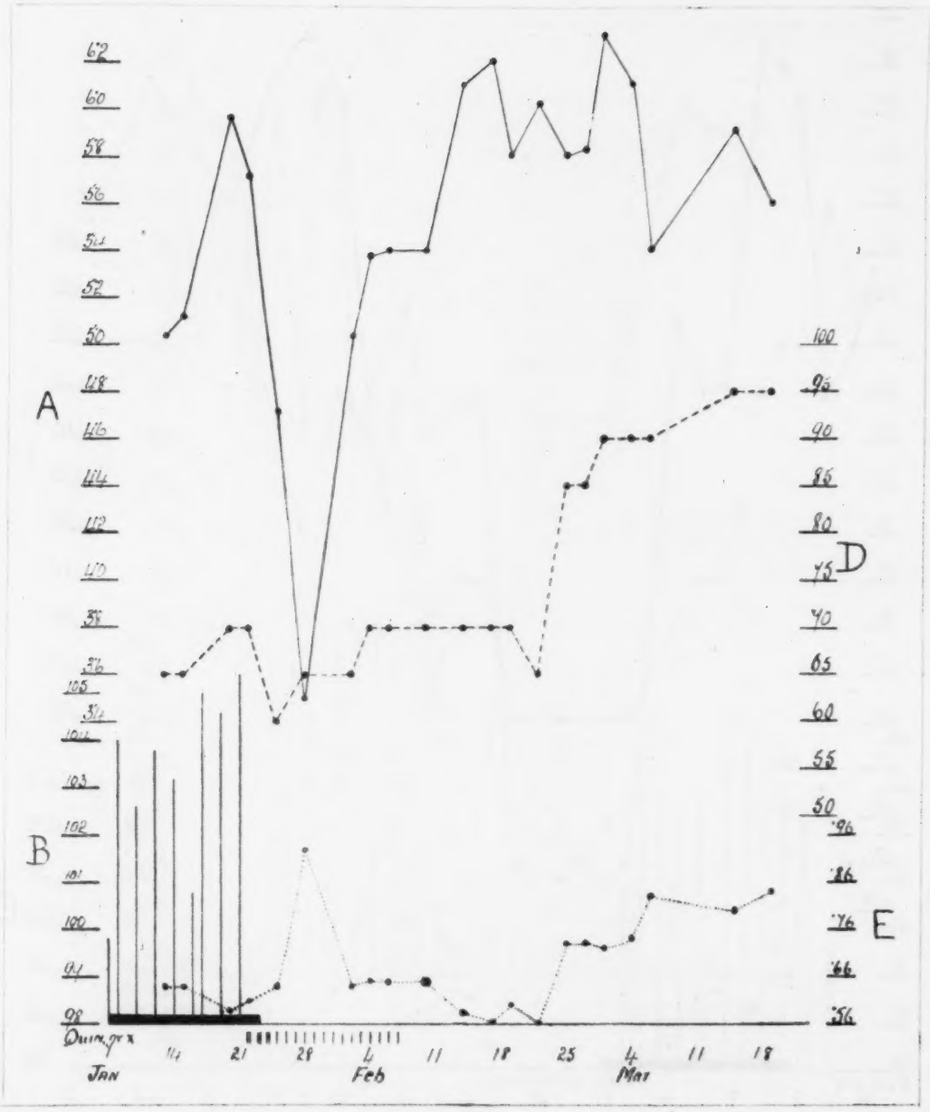

Case No. 4

THE EFFECT OF ANCYLOSTOME, ASCARIS, AND TRICHURIS INFECTIONS ON THE HEALTH OF THE WEST AFRICAN NATIVE

BY

R. M. GORDON

(*From the Sir Alfred Lewis Jones Research Laboratory, Freetown, Sierra Leone*)

(*Received for publication* 28 *October*, 1925)

PLATE VII

TABLE OF CONTENTS

I. INTRODUCTION

The limitations of the work must first of all be made clear.

1. It deals solely with the West African male native, as studied at Freetown, and any conclusions drawn apply only to this race.

2. It is concerned only with the effects of the infection on the individual; it is not concerned with the importance of the infected individual as a propagator of the disease.

3. The lesions and symptoms produced by migrating larvae are considered to be a separate subject and are not discussed.

4. The effect of treatment is not considered; whereas this subject is obviously one of great importance, it appears to the writer that the first and most important consideration is the effect, if any, of ancylostome infection on different races of mankind.

5. No distinction is drawn between infection with *A. duodenale* and *N. americanus*. Darling and others (1920) and Darling (1922) show that *A. duodenale* is more important as a producer of anaemia than an equal infection with *N. americanus*. Adler (1925) gives the proportion of *A. duodenale* to *N. americanus* in Freetown as 1 : 10.

Malaria and ankylostomiasis are usually accepted as the most important diseases affecting natives in West Africa, and certainly entail a greater expenditure of money than any other two diseases. The effects of Malaria are definite, and the pathological changes which it produces in the individual can be demonstrated and classified, while the value of its eradication is too obvious to need argument. The position as regards ankylostomiasis appears to be entirely different ; the causal organism and its life cycle are established, and the value of its eradication appears generally accepted, but the effect of the worm on its host and the pathological lesions produced by it seem to be subjects evoking the widest differences of opinion, ranging from those who regard ankylostomiasis in general as having little effect on the human host, to the other extreme, which considers that any infection, however light, is responsible for illness of the individual, and calls for immediate treatment ; between these two extremes are to be found numerous observers who consider that a certain concentration of worms must be present in the gut before any symptoms appear. Unless it is conceded therefore, that any infection, however small, is pathogenic, it is obvious that any attempt to define the pathogenicity of ankylostomiasis must include a statement showing the degree of infection of the individuals considered ; it is doubtful if any value can be attached to mere comparisons of infected and uninfected individuals, such comparisons being frequently made and often illustrated with photographs showing poorly developed individuals suffering from ancylostome infection as diagnosed by the finding of ova (the number not being stated) in the stool, and well-developed, athletic-looking individuals free from infection. The two photographs accompanying the present article represent, in the one case, six boys selected at random from amongst the heaviest infections, and in the other case, six boys selected at random from amongst the negative or lightly infected group ; if anything, it is the heavily infected group which appears to show the best physique. In the enormous bibliography of hook-worm

disease definite figures of the degree of infection of the cases considered are surprisingly few, this lack of figures being, of course, largely due to the fact that until the publication of Stoll's (1923) method of estimating the number of ova in a given sample of faeces, there was little uniformity of opinion as to what constituted a ' light ' or ' heavy ' infection, and it is probably this lack of uniformity of opinion that has led to the surprising diversity of statements concerning the pathogenicity of ankylostomiasis.

Another difficulty encountered is that the literature dealing with ankylostomiasis appears almost entirely to ignore concomitant infections with other gut helminths, even when the pathogenicity of such helminths is admitted by the authors of the publication.

During the first part of the present work, which consisted in examining prison cases, the writer was impressed with the high proportion of Ancylostome cases which were also infected with Ascaris or Trichuris or both ; in subsequent examinations, therefore, a count was kept of these ova, and a classification made of the cases on the same lines as in the ancylostome work. Infection with the larvae of *Strongyloides stercoralis* was also common in Freetown, as noted by Maplestone (1924) ; they are not recorded here owing to the difficulty of estimating the degree of infection, which varied enormously with the consistency of the stool. Ova of a cestode (probably *T. saginata*) and on one occasion those of *S. mansoni* were also noted ; both infections were rare and when seen the ova were too few in number to be worth estimating.

Granted that ankylostomiasis is pathogenic, there still remain great differences of opinion as to the manner in which this pathogenicity manifests itself in the individual and what lesions, if any, the worm produces in the gut. Thus a perusal of the ' Rockefeller Bibliography of Hookworm Disease ' (1922) shows that it tabulates articles on almost every conceivable sign and symptom ranging from arthritis to night-blindness. The present writer attempted to compile a table summarising the views of modern authorities on the subject, but it was found that such a table became hopelessly unwieldy, as it had to include columns for almost every system and organ of the body. Most, though not all, authorities are, however, agreed *that ancylostome infection adversely affects the host by producing* (1) *Anaemia*, (2) *Poor physique*, (3) *Mental dullness*, (4) *Lack of*

energy. It is with these four points that the present paper, which deals with 137 natives, of whom 114 (83 per cent.) were infected, is concerned.

The number of cases dealt with is small and it may at first seem unnecessary to add them to the already overburdened literature on the pathogenicity of ankylostomiasis, but the information concerning these cases has been made as comprehensive and as exact as possible, whereas, as already stated, by far the greater proportion of published literature on this subject lacks figures showing the degree of infection of the cases under consideration and abounds in statements associating this or that symptom with ancylostome infection, such statements being unsupported by any proof except the finding of an unestimated number of ancylostome ova in the stool of the patient. It is interesting in this connection to consider the following statements. Stephens (1916), quoting Löbker and Bruns (1906), writes ' Whilst up to modern times it has been generally maintained that the great majority of worm diseases cause more or less marked symptoms, the exact investigations of the last few years have made it plain that the great majority of people with worms are not only perfectly healthy, but the most careful clinical observations show no single sign of any ill-effect of the intestinal parasites on the health of the host (Löbker and Bruns).' Clayton Lane (1917) points out that the reference date of Löbker and Bruns is 1906 and dismisses the whole statement as being out of date ; referring to the Rockefeller Sanitary Commission for the Eradication of Hookworm Disease and the Rockefeller International Health Commission, Lane continues as follows : ' It is obvious that the opinions based on this enormous experience, which in the five years of the Sanitary Commission's existence, covered over 1,300,000 persons, carries a weight borne by those of no other person or scientific body in the world ; and that should any individual elect to differ, the onus of fully justifying his own attitude must lie with himself.' Such a statement as this has the natural effect of deterring the individual observer from adding his small quota to so vast an array of figures ; no one who has studied the literature of the Rockefeller Commission can but be impressed with the magnificent work published and the splendid results obtained by this body of investigators. Yet at the time of Lane's paper (1917) the present

writer is unaware of any paper published by the Rockefeller Commission which dealt with the degree of infection of the persons considered, except in a few instances where a rough comparison is made by the general appearance of the number of ova in the stool ; the whole of the 1,300,000 cases referred to are only considered as a comparison of infected and uninfected individuals ; thus Strong (1916) investigated the effects of ankylostomiasis on the physique and mentality of 115 school children and drew the following conclusions.

' (1) *Our figures show that hookworm disease interferes with physical development. Treatment alleviates this condition to a considerable extent. Apparently young children can regain most of the physical conditions, if not all, which they have lost due to the infection.* But the data do also very strongly suggest that the severer the infection and the longer it persists, the less likely it is that the child will ever reach his normal physical development.' He draws the following conclusions as a result of the mental tests. ' The figures show, then, that hookworm disease unmistakably affects mental development. *Treatment alleviates this condition to some extent but it does not, immediately, at least, permit the child to gain as he would if he had not had the disease. And the figures apparently further show that prolonged infection may produce prolonged effects upon mentality—effects from which the individual may never entirely recover.*' No estimation was made in these cases of the concentration of ova in the stools or the number of worms in the intestines of the children. The consideration of these cases, therefore, resolves itself essentially into a comparison of infected and uninfected cases.

Our present knowledge shows that such a comparison is liable to very wide error. To quote one instance only : Hill (1923) records 282 cases, of whom 142 with 1 to 2,099 ova per gm. showed no symptoms, while 57 with 2,100 to 5,099 only showed very slight and indefinite symptoms. A few months prior to the publication of this paper, Lane (1923b) wrote as follows : ' It is at least certain that there is a growing mass of evidence that the so-called carrier is improved in health and working power by disinfestation, and I know of no published evidence suggesting that there is any limit below which infestation is immaterial. Statements of personal belief on this matter appear misplaced. The fact seems to be that there is

no satisfactory evidence, either for or against the belief that the lightest infestations are immaterial to their host.' This statement would appear to the present writer to be a very fair summary of the state of affairs at the time the paper was published, except that the latter portion of the statement seems to negative the value of the remarks as regards the so-called carrier being improved in health and working power by disinfestation, and would also appear to indicate that Lane has considerably modified his previous views, as in 1919 he states : 'Each year adds to the accumulated facts indicating that even light infections are a definite handicap to growth in wisdom and stature, and to the full possession of that modicum of health and wealth which makes life worth living.'

A search of the literature has shown that the following are the more important papers dealing with the effects of ankylostomiasis on the host when the degree of infection is approximately known. (1) Darling, Barber and Hacker (1920) ; (2) Darling and Smillie (1921) ; (3) Smillie (1922) ; (4) Hill (1923) ; (5) Cort, Payne and Riley (1923) ; (6) Mhaskar and Kendrick (1923) ; (7) Cort (1924) ; (8) Mhaskar (1924) ; (9) Chandler (1925) ; (10) Stoll and Tseng (1925). The work of these writers on the pathogenicity of ankylostomiasis is almost entirely concerned with the question of anaemia, and there appears to be a great need of further investigation as to its effects on the health and mentality of different native races.

It will be noted that the above summary refers only to ankylostomiasis ; the writer is unaware of any publication dealing with the pathology of Ascaris or Trichuris infection based on a knowledge of the intensity of the infection in the individuals concerned.

II. CASES AVAILABLE, CLASSIFICATION OF CASES, COLLECTION OF MATERIAL

Only cases which were under constant supervision and discipline were selected. They were chosen from amongst three sections of the native male community in Freetown. (1) 49 youths aged 10 to 22 (average age 18) attending school, the majority as boarders ; (2) 40 City Police of all ages from 23 to 50 ; (3) 48 gaol prisoners of all ages from 17 to 49. One hundred and thirty-seven cases

were thus examined, the work occupying about four hours daily for a period of eight months.

In every case the examination, as regards physique, mentality and energy, was carried out by the officer in charge of the institution concerned, according to a fixed scheme previously carefully discussed and agreed upon between the officer and the Laboratory. In order to avoid any bias that might result from any previous knowledge, the officers in charge of the institutions did not know the degree of infection of the inmates, and the Laboratory was unaware of the classification of the cases it was examining. The haemoglobin percentage, as shown by a Talquist scale, was estimated by the writer who was not aware of the identity of the particular case he was at the time investigating. In addition to the haemoglobin estimation, each case was examined as regards three other categories : (1) Physique and general fitness, (2) Mentality, and (3) Energy, and placed in order of merit, in an *A*, *B* or *C* class in each of these three categories.

The physical examination requires no special comment ; it was not necessarily a medical examination (though the doctor's report was usually available) but consisted in placing the native in class *A*, *B*, or *C* according to his general physique and fitness when seen stripped.

The mental examination was not directed to ascertain how much the individual knew, but rather to discover his mental alertness and ability to learn ; thus a boy at the head of his class might be placed in category *C* because he had gained his position at the head of the class by remaining behind when brighter boys had been moved on. The mental classification was comparatively simple in the case of the boys and police who were being regularly taught and questioned, but in the case of the gaol prisoners, it had to consist of an examination of the man's mental ability as judged by the answers he gave to a series of simple questions.

Energy was defined as the keenness with which an individual attempted any mental or physical task allotted to him ; it was frequently found that this classification gave very different results from the other two ; thus a native might be classed as physically poor (*C*) and mentally dull (*C*), but as regards energy very good (*A*) because, though his ability to perform any task, whether physical

or mental, allotted to him, was bad, yet the energy he showed in trying to perform the task was excellent.

Strong (1916) has published a long and very carefully detailed account of his investigations on the effects of ankylostomiasis on the physique and mentality of 115 school children living in a ' hookworm infected county.' He divided the children into five groups according to whether ' (1) They were not infected (Group A) ; (2) They were infected but not treated (Group B) ; (3) They were infected and later cured (Group C) ; (4) They were infected and treated but not completely cured (Group D) ; or (5) They were infected and treated but the final condition of their infection could not be determined (Group E).'

The tests applied to these groups were extremely ingenious and interesting, but appeared too complex for use in a native community such as Freetown. The question of Strong's conclusions from these tests has already been dealt with (see Introduction).

The classification having been completed, the case was issued with a faeces container marked with his number and, as a rule, the specimen was passed under the observation of an individual appointed for the purpose ; the specimen was then dispatched to the Laboratory and examined according to the technique described later. The information regarding the case was therefore tabulated on two forms, one form being filled in by the Laboratory and the other by the person in charge of the school, barracks, or gaol, the two portions being compared together only when the work was completed. In the case of the 48 gaol prisoners only ancylostome ova were counted and facilities for haemoglobin estimation were not available ; in the case of the 40 city police and the 49 youths attending school, Ascaris and Trichuris ova were counted in every case, and in 84 of the 89 cases the haemoglobin percentage was also estimated.

III. TECHNIQUE OF ESTIMATING THE NUMBER OF OVA IN THE FAECES

Stoll (1923) published a technique for counting hookworm eggs in faeces, and in 1924, he published a further paper in the conclusions of which he states ' A relationship of approximately 1 : 2 : 4 is found to exist in general between the weighed amounts of formed, mushy (unformed), and diarrhoea faeces, passed per day. This affords an

easy interpolation by which to bring counts made on faeces of any of the three categories to a similar plane, the basis of formed faeces, so that they can be compared *inter se*.' The reader is referred to these two papers for details of the technique, which was exactly adhered to except for the following trifling modifications and additions. (*a*) Stoll balances the container and its faeces on the scales and removes 3 gm. into a large test-tube containing three glass beads and 45 c.c. of $\frac{N}{10}$ NaOH. The writer found it simpler and less messy to stir thoroughly the specimen of faeces and weigh out 3 gm. into a small metal container previously balanced on the scales, and then slide the metal dish, containing the 3 gm. of faeces, into the large test-tube and add 45 c.c. of $\frac{N}{10}$ NaOH and three glass beads. The metal dish aids greatly in rapid emulsification of the faeces when shaking the tube, and is also very convenient for dealing with liquid faeces. The dish referred to is made of the thin tin used in sealing boxes of cigarettes sent to the tropics ; the tin should be cut into a square and the four corners inbent so as to form a rectangular water-tight trough measuring about 2 in. $\times$ $\frac{3}{4}$ in. $\times$ $\frac{1}{2}$ in. (*b*) Stoll says the diluted faeces ' was immediately sampled with a pipette graduated at 0.15 c.c.' It will be found in practice that in some cases faecal debris adheres to the outside of the pipette and interferes with accuracy by draining into the fluid which is being discharged on to the counting glass ; in order to avoid this error it is advisable to draw up fluid past the 0.15 c.c. mark, rapidly wipe the outside of the pipette with a wisp of wool, discard the excess of fluid and discharge the remaining 0.15 c.c. on to the counting glass. It is necessary to perform this operation very rapidly in order to avoid sedimentation occurring. (*c*) Stoll measures 0.15 c.c. of the diluted faeces on to a large slide and covers this with a single 22 $\times$ 40 mm. coverslip. The writer found it more convenient to use three amounts of 0.05 c.c. and count each separately. (*d*) A small point, but one which, if neglected, interferes with accuracy, is that the surplus uncovered fluid lying along the edge of the coverslip should be first examined, otherwise the rapid drying up which occurs in the tropics will render the counting of ova difficult. (*e*) At the commencement of the work it was found that certain bodies, probably derived from some

vegetable in the native dietary, imitated unfertilised Ascaris ova with such extreme fidelity, both as regards size and morphology, that they necessitated careful examination with the high power in order to differentiate them from ova ; it was therefore decided to include only ' fertile ' Ascaris, Ancylostome or Trichuris ova in the counts. (*f*) Chandler (1925) advocates examining uncovered preparations, as by this method one can blow aside obscuring flocculi of undissolved faecal debris, while doubtful egg-like objects can be ver fied by blowing on the fluid and causing them to roll about. The writer tried this method prior to the publication of Chandler's paper and abandoned it because it was found that any current of air occurring in the laboratory caused the ova in the fluid to move about and lose their position in the field which was being counted.

IV. ACCURACY OF STOLL'S METHOD AND ITS VALUE IN COMPARING THE DEGREE OF INFECTION IN DIFFERENT INDIVIDUALS

Stoll (1923), when describing his method of estimating the number of hookworm eggs in faeces, claimed that this technique was ' accurate to within 10 per cent.', while Maplestone (1924), who tested the accuracy of the method by control counts of ova made with saturated salt solution, and cultures of larvae made from the same faeces sample, came to the conclusion that the method was ' not accurate to within 10 per cent.'

In order to compare together the ovum content of stools of different consistencies, Stoll (1924) advocates the taking of a formed stool as a standard and multiplying the ovum content of a mushy stool by two and a liquid stool by four (this being the method adopted in the present work) ; Chandler (1925) regards mushy stools as normal for the Indian native, and therefore divides the results obtained from formed stools by two and multiplies those of liquid stools by two ; whatever the accuracy, therefore, of any technique for estimating the number of ova in a single given sample of faeces, it is obviously absurd to discuss the finer points of accuracy of such a technique when applied to the estimation of the average number of ova in a series of stools varying in consistency, for the definitions ' formed ' ' mushy ' (or ' semi-solid ') and ' liquid ' are not fixed

definitions, and an examination of even as few as fifty specimens will convince any worker on this subject that every variation between these standards is to be found, where one observer will define a stool as mushy and multiply his result by two, another will call it liquid and multiply his result by four. A clear-cut distinction must, therefore, be drawn between estimating the number of ova in a given sample of faeces, and the comparing together of the average number of ova passed by different individuals on different occasions ; it is obvious that the first can be performed to any degree of accuracy if sufficient time and care are expended ; thus Stoll's method admittedly does not detect the presence of ova in the stool if less than 100 per gm. be present. Therefore all single worm infections will be missed. Now Lane's (1923-1925) method will detect less than this concentration and clearly, therefore, his method is more accurate and, therefore, more suitable for such an estimation. The present work, however, is not concerned with such an estimation, but is concerned with the comparison of the ovum content of the stools of different individuals on different occasions ; now the ovum content of such stools may vary according to the quantity of faeces passed (presumably the less food taken the smaller the quantity of faeces and the greater the concentration of the ova), the consistency of the faeces, and the fecundity of the worms. When such a number of uncontrolled factors exist the more minute points of accuracy are of little importance ; what is required is a method whereby negative (by negative is meant less than 100 ova per gm.), moderate, and heavy infections can be compared together, and for this purpose Stoll's technique seems well adapted. That such comparative accuracy is obtainable by Stoll's method appears to be proved by the figures given in Table I. It is of interest to note that the counts of Ascaris and Trichuris infections do not correspond nearly as closely as do those of anclystome infections, a possible explanation being that fewer worms are present in the two former infections and that the variations in the fecundity of the worms are, therefore, more clearly noticeable.

For further particulars regarding the comparative accuracy of Stoll's (1923) and Clayton Lane's (1923-1925) method, the reader is referred to articles by Sweet (1924) and Chandler (1925b).

Tables Ia, Ib, Ic.

Showing the number of ova actually counted in 0·01 gm. of faeces in the same individual on three occasions, amongst a series of 114 positive Ancylostome cases, 16 positive Ascaris cases, and 39 positive Trichuris cases. Solid specimens are shown by black figures. When a specimen was 'mu' 'semi-solid' the result has been × 2, and when liquid × 4. For the purpose of reference the numbers given to the cases have been adhered to throughout the tables and

Table Ia.—One hundred and fourteen Positive Ancylostome Cases.

Note.—The occurrence of a decimal point in a few of the cases is due to the figure being the average of a series of counts on the same specimen.

Case	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1st examination...	32	**0**	**3**	**24**	**50**	**38**	**2**	**6**	0	**3**	**0**	**13**	**4**	26	**13**	**3**	2	**3**	**9**	**7**	**10**	**8**	**9**	**17**	**54**	**2**	**11**	**3**	**5**
2nd examination	32	**2**	**1**	**44**	**67**	**38**	**1**	**3**	**1**	**4**	**4**	**11**	**1**	**12**	8	2	0	**1**	**8**	**8**	**7**	**6**	**29**	**23**	**50**	**4**	**9**	**8**	**4**
3rd examination	28	**0**	**2**	**49**	**49**	**30**	**1**	**4**	**1**	**3**	**2**	**9**	**6**	28	**35**	**0**	6	2	**9**	6	12	**6**	**4**	**18**	**53**	**5**	**9**	**17**	**5**

Case	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58
1st examination...	**4**	**6**	**26**	**203**	**41**	128	0	**122**	**154**	**21**	**16**	**12**	**15**	**304**	**272**	**50**	**10**	**196**	**53**	**38**	**102**	**75**	**30**	**97**	**122**	**92**	**15**	**65**	**27**
2nd examination	2	**9**	**19**	**122**	**48**	**140**	**0**	**110**	**127**	**27**	**17**	**18**	**22**	**313**	**295**	**42**	**4**	**62**	**48**	**42**	**70**	**111**	**43**	**81**	**122**	**114**	**13**	76	**53**
3rd examination	0	**5**	**18**	**143**	**43**	**144**	**4**	**61**	**117**	**37**	**22**	**17**	**20**	**488**	**126**	**26**	**13**	**135**	**50**	**77**	**109**	**130**	**40**	**70**	**136**	**130**	**11**	**70**	**31**

Case	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87
1st examination...	**1**	**175**	**16**	**40**	2	**5**	**21**	**3**	**5**	8	**0**	**5**	**271**	**1**	**43**	**4**	**29**	128	**66**	13	**2**	23	0.5	5	**4.5**	**46**	**9.5**	**4.5**	**53**
2nd examination	**4**	**155**	**17**	**25**	**3**	2	**11**	**3**	**7**	**13**	**0**	**2**	**188**	**7**	**42**	2	**37**	178	56	**15**	**8**	7	·0	**6**	4	**51**	32	6	**39**
3rd examination	10	**79**	**13**	**29**	**0**	**3**	**19**	**0**	**4**	**12**	**1**	3	**174**	**5**	**57**	**4**	**34**	96	**55**	12	**3**	22	3	16	**5**	**88**	32	**5**	**50**

Case	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114
1st examination...	**23**	**70**	5	**0**	22	**0**	**2**	10	**0.5**	0	4	**23**	**92**	**1**	**27**	**6**	**79**	**14**	19	**2**	**3**	**4**	30	1	**34**	1412	**141**
2nd examination	20	**80**	**15**	**0**	16	0	2	4	**1**	**2**	**13**	**43**	**165**	**2**	**76**	8	**77**	**13**	**33**	1	**12**	**7**	39	**2**	20	1070	**210**
3rd examination	**29**	**31**	12	16	**54**	**16**	**1**	4	**0**	**0**	32	**25**	128	**1**	...	0	**65**	**19**	...	2	**5**	8	...	4	18	...	**182**

Table Ib.—Sixteen Positive Ascaris Cases.

Case	3	115	116	30	38	43	51	57	117	64	65	66	72	74	119	75
1st examination	**44**	**115**	48	**164**	**507**	**96**	**31**	**102**	**61**	**5**	**34**	**192**	**63**	**218**	**21**	**16**
2nd examination	**12**	**103**	**53**	214	**317**	**103**	**42**	48	88	2	**37**	**126**	**134**	92	**31**	**16**
3rd examination	**30**	**88**	**27**	144	**455**	**291**	**44**	**124**	**134**	**4**	**54**	**214**	**64**	**275**	**30**	**47**

Table Ic.—Thirty-nine Positive Trichuris Cases.

Case	1	120	6	7	8	11	12	14	15	17	19	20	21	22	23	24	121	26	122	31
1st examination	0	**1**	2	**1**	**1**	**0**	**0**	8	**10**	**1**	**1**	**0**	**3**	**4**	**0**	**0**	0	**6**	**2**	**5**
2nd examination	0	**0**	**2**	**0**	**1**	**2**	**3**	**1**	4	**1**	**0**	**19**	**1**	**2**	**1**	**2**	**2**	**0**	**0**	**0**
3rd examination	2	**0**	**2**	**2**	0	**0**	**1**	2	**16**	0	**1**	48	4	**3**	0	**0**	**1**	**1**	**2**	**3**

Case	35	38	43	49	123	60	117	62	63	64	68	124	125	69	73	74	119	75	76
1st examination	0	**0**	**52**	**1**	**3**	**1**	**3**	**0**	**0**	**3**	0	**1**	**3**	**1**	**5**	**14**	**5**	**0**	2
2nd examination	2	**1**	**48**	**1**	2	0	..	0	0	..	0	3	2	0	0	12	4	0	2

V. EFFECTS OF ANCYLOSTOME, ASCARIS, AND TRICHURIS INFECTIONS ON THE (*A*) HAEMOGLOBIN PERCENTAGE, (*B*) PHYSIQUE AND GENERAL FITNESS, (*C*) MENTALITY, (*D*) ENERGY, AND (*E*) URINE, OF WEST AFRICAN NATIVES

In the results which follow, the degree of infection is expressed as the average number of ova per gm. of faeces, this figure being the mean of three Stoll counts on each individual case, except that five cases amongst the 137 examined for ancylostome infection, and two each amongst the eighty-nine Ascaris and eighty-nine Trichuris series were only examined on two occasions, the natives concerned having left the Institution before the third examination was completed. The counts were usually made at intervals of four to seven days, but occasionally longer periods intervened. The expression 'average number of ova per gm. of faeces,' when applied to a number of cases constituting a group or class, includes negative cases, that is to say, it is the figure arrived at by adding together the average number of ova per gm. of faeces of each member of the group and dividing the result by the total number of individuals in that group; the same rule holds good for the heading 'average number of ancylostomes per individual.' The figures for the number of ancylostomes are, of course, only roughly approximate, but they are included in the tables as they would appear to give a more concrete idea of the degree of infection; the estimation of the number of ancylostomes is based on the supposition that every forty ova per gm. of faeces represents one adult female worm; this relation between ova per gm. and parent worm is given by Stoll (1923b) as 44 to 1, by Darling (1922) as 22 to 1, by Lane (1923) as 33 to 1, and by Davis (1924) as 85 to 1. To this figure must be added the proportion of male worms which is here estimated as two males for every three females (see Darling, Barber and Hacker (1920), Stoll (1923b), Adler (1925)). The final formula is, therefore, $\frac{x}{40} + \frac{2}{3}\left(\frac{x}{40}\right)$ where x is the number of ova per gm. of faeces. Figures of the number of Ascaris and Trichuris present in the gut are omitted, as there appears to be no authoritative statement as regards the average daily egg production of these two species, except those of Davis (1924) who, as the result of the examination, and subsequent treatment, of

sixteen positive Ascaris cases computes the average number of eggs per female, per gm. of faeces, to be 3,466, and Moosbruger, as quoted by Brumpt (1922), who, as the result of an autopsy, gives the Trichuris figure as seven ova per female per gm. of faeces.

The influence of Ancylostome, Ascaris and Trichuris infections will be considered as regards their effects on five conditions. (A) Haemoglobin percentage. (B) General physique and fitness. (C) Mentality. (D) Energy. (E) Urine.

(A) *Haemoglobin percentage.* It will be seen that Table II lends no support to the commonly accepted view that Ancylostome infection tends to lower the haemoglobin reading ; nor do Ascaris or Trichuris infections appear to have any influence, for it can be seen that the group with the higher haemoglobin reading actually contains a slightly higher average degree of infection than the group with the lower haemoglobin reading. It is perhaps unfortunate that the haemoglobin readings in the two groups approximate so closely, but this was unavoidable as the haemoglobin readings in all the West Africans examined fell between 90 and 70 per cent.

TABLE II.

Showing the percentage of individuals infected with Ancylostome, Ascaris, and Trichuris, and the average degree of infection, in each of two groups of West African natives classified according to the haemoglobin reading.

Groups based on Haemoglobin per cent.	Number of cases examined	Percentage infected with			Average number of ova per gm. of faeces			Maximum number of ova per gm. of faeces			Compute average number Ancylostom per indivi
		Ancylos-tome	Ascaris	Trichuris	Ancylos-tome	Ascaris	Trichuris	Ancylos-tome	Ascaris	Trichuris	
A 81–90% Hb	57	86	19	47	3,670	1,878	125	21,100	42,630	2,230	19
B 71–80% Hb	25	84	16	36	2,890	1,947	118	23,100	19,500	933	12

The question now arises whether intense infection produces any marked change ; with the object of investigating this point the ten heaviest infections in the case of each worm are set forth in Table III.

TABLE III.

Showing the ten heaviest Ancylostome, Ascaris, and Trichuris infections observed amongst West African natives, and the haemoglobin reading for each case.

ANCYLOSTOME				ASCARIS			TRICHURIS		
Case	Haemoglobin per cent	Number of ova per gm. of faeces	Computed number of Ancylostomes	Case	Haemoglobin per cent.	Number of ova per gm. of faeces	Case	Haemoglobin per cent.	Number of ova per gm. of faeces
44	70	23,100	962	38	80	42,630	20	80	2,230
71	80	21,100	879	74	75	19,500	15	80	1,000
33	80	15,600	650	66	75	17,733	73	75	933
35	85	13,700	570	30	85	17,400	74	75	900
60	90	13,600	567	115	80	10,300	60	90	460
76	85	13,400	558	117	80	9,430	119	75	400
38	80	13,200	550	57	85	9,130	14	80	360
47	70	13,100	546	72	75	8,700	49	90	330
54	90	12,660	527	116	80	4,260	22	85	300
55	70	11,200	466	65	85	4,160	124	85	266

It will be seen from Table III that, broadly speaking, two-thirds of the heaviest infections in the case of each worm fall into the higher haemoglobin group; moreover, if we consider the haemoglobin content of those natives who were uninfected, we find that of the eighty-two cases in which haemoglobin readings were made, twelve were negative as regards ancylostomes, and of these eight fell into the higher haemoglobin group and four into the lower. Sixty-seven were negative as regards Ascaris, and of these forty-six fell into the higher haemoglobin group and twenty-one into the lower. Forty-six were negative as regards Trichuris, and of these thirty fell into the higher haemoglobin group and sixteen into the lower. Thus it appears that roughly two-thirds of the heaviest infections and two-thirds of the negative cases fell into the higher haemoglobin group, and this figure corresponds with the relative size of high and low haemoglobin groups amongst the total eighty-two natives examined, that is, 57 to 25.

From these facts it seems clear that there is no correlation between intensity of infection in the individual and the haemoglobin reading. It might, of course, be argued that all the natives under consideration exhibited some degree of anaemia ; this may be so but it must be borne in mind that in none of the eighty-two West African natives—whether infected or uninfected—chosen at random, was the haemoglobin reading more than ninety, so that in any case, if the readings in this series were less than normal, this anaemia cannot be due to any of the three worms under consideration.

Conclusions regarding the influence of Ancylostome, Ascaris, and Trichuris infections on the haemoglobin percentage of eighty-four West African Natives.

1. A group of individuals with a low haemoglobin percentage does not show a greater percentage of infected cases than a group with a higher haemoglobin percentage.

2. A group of individuals with a low haemoglobin percentage does not show a greater average degree of infection than a group with a higher haemoglobin percentage.

3. Individuals with a high degree of infection do not necessarily show a low haemoglobin percentage.

This tolerance, so far as ankylostomiasis is concerned, would appear to be shared by some, at any rate, of the Indian races. Thus Mhaskar and Kendrick (1923), working in the tea estates of Madras, report as follows :—' There is no correlation between the haemoglobin average and the number of hookworms harboured ; the presence of anaemia is not necessarily a sign of heavy infection.' Chandler (1925), using Stoll's technique, writes : ' In a study of 100 individuals in the Alipore Central Jail, Calcutta, sixty-seven of whom were infected with hookworm, but only six of whom had more than 1,000 eggs per gm. of faeces, no differences in haemoglobin percentage between the infected and uninfected individuals could be found.'

On the other hand, Stoll and Tseng (1925), working with Chinese cases, trace a definite connection between the number of ancylostomes harboured and the degree of anaemia ; it is important to note, however, that the haemoglobin percentage of sixty-four ancylostome-free cases only averaged 66.7 per cent. and concerning these they write : ' The malaria is held to account for the low average haemoglobin of the hookworm negatives, and probably also influenced the degree of anaemia in the hookworm positives.'

(B) *Physique and General Fitness.* From a consideration of the figures in Table IV it is clear that the percentage of positive ancylostome cases is approximately equal in all three groups; on the other hand, there appears at first sight to be a very definite relationship between the average degree of infection and the physical standard of the group in which the cases occur; on consulting the column of maximum infections, however, it will be seen that the maximum infections occurring in Group B and Group C are much higher than that in Group A.

TABLE IV.

Showing the percentage of individuals infected with Ancylostome, Ascaris, and Trichuris, and the average degree of infection, in each of three groups of West African natives classified according to their physique and general fitness.

Groups based on physique and general fitness	Number of cases examined	Percentage infected with			Average number of ova per gm. of faeces			Maximum number of ova per gm. of faeces			Computed average number of Ancylostomes per individual
		Ancylostome	Ascaris	Trichuris	Ancylostome	Ascaris	Trichuris	Ancylostome	Ascaris	Trichuris	
A (Good)	For Ancylostomes only—32 For Ancylostomes, Ascaris and Trichuris—50	84	22	44	2,619	2,418	102	15,600	42,630	933	109
B (Moderate)	For Ancylostomes only—5 For Ancylostomes, Ascaris and Trichuris—31	83	16	39	4,381	1,651	335	36,830	17,733	7,800	183
C (Bad)	For Ancylostomes only—11 For Ancylostomes, Ascaris and Trichuris—8	84	0	62	9,420	0	340	124,000	0	2,200	392

It is obvious that when one is dealing with a comparatively small number of cases a single pre-eminently heavy infection may be sufficient to raise to a considerable extent the average degree of infection of the whole group; and on enquiring into the question it was found that the maximum infections in Group B and Group C, shown in Table IV, were in fact outstanding, as the next highest infection in Group B was 23,100, and in Group C 17,760. Turning to a consideration of the Ascaris and Trichuris infections it was

similarly discovered that in each case there was a pre-eminently high infection, viz., 42,630 Ascaris ova per gm. in Group A, and 7,800 Trichuris ova in Group B. A truer picture is, therefore, obtained by omitting these predominantly high infections, and this is done in Table IVA.

TABLE IVA.

Table IV modified by the omission of the four predominantly high infections.

Groups based on physique and general fitness	Number of cases examined	Percentage infected with			Average number of ova per gm. of faeces			Maximum number of ova per gm. of faeces			Compu... averag... numbe... Ancylos... per indiv...
		Ancylos-tome	Ascaris	Trichuris	Ancylos-tome	Ascaris	Trichuris	Ancylos-tome	Ascaris	Trichuris	
A (Good)	For Ancylostomes only—32 For Ancylostomes, Ascaris and Trichuris—49	84	20	44	2,619	1,597	102	15,600	19,500	933	100
B (Moderate)	For Ancylostomes only—5 For Ancylostomes, Ascaris and Trichuris—30	83	16	37	3,453	1,651	86	23,100	17,733	1,000	141
C (Bad)	For Ancylostomes only—10 For Ancylostomes, Ascaris and Trichuris—8	83	0	62	3,054	0	340	17,760	0	2,200	12

It is seen from Table IVA that no definite correlation exists between the physical standard of a group and the percentage of infected cases, or the degree of infection occurring in that group.

Turning to the subject of the effect of intense infections, Table IV shows that the total number of cases examined for ancylostomes was 137; of these eighty-two (60 per cent.) fell into Group A, thirty-six (26 per cent.) fell into Group B, and nineteen (14 per cent.) into Group C. Eighty-nine natives were examined for Ascaris and Trichuris infections and were found to be distributed amongst the three groups in the following proportions; Group A, fifty (56 per cent.), Group B, thirty-one (35 per cent.), and Group C, eight (9 per cent.). It is now necessary to consider the distribution of the ten heaviest infections with each species of worm amongst the three groups, and to compare this distribution with that of the uninfected cases.

TABLE V.

Showing the ten heaviest Ancylostome, Ascaris, and Trichuris infections observed amongst West African natives, and the standard of physique and general fitness for each case.

ANCYLOSTOME			ASCARIS			TRICHURIS		
Case	Standard of physique	Number of ova per gm. of faeces	Computed number of Ancylostomes	Case	Standard of physique	Number of ova per gm. of faeces	Case	Standard of physique	Number of ova per gm. of faeces
113	C	124,000	5,167	38	A	42,630	43	B	7,800
43	B	36,830	1,537	74	A	19,500	20	C	2,200
44	B	23,100	962	66	B	17,733	15	B	1,000
71	B	21,100	879	30	A	17,400	73	A	933
114	C	17,760	740	43	B	16,630	74	A	900
33	A	15,600	650	115	B	10,300	60	A	460
35	B	13,700	570	117	A	9,430	119	A	400
60	A	13,600	567	57	A	9,130	14	A	360
76	A	13,400	558	72	B	8,700	49	B	330
38	A	13,200	550	65	B	4,260	22	A	300

From Table V it can be seen that the ten heaviest infections with Ancylostome, Ascaris, or Trichuris, are in each case distributed according to the size of the group. Amongst the ten most intense Ancylostome infections 40 per cent. occur in Group A, 40 per cent. in Group B, and 20 per cent. in Group C; an examination of twenty-three natives who were not infected with Ancylostomes showed that 61 per cent. fell into Group A, 26 per cent. into Group B, and 13 per cent. into Group C. Of the ten heaviest Ascaris infections 50 per cent. occurred in Group A, and 50 per cent. in Group B; and of the seventy-three cases negative for Ascaris, 53 per cent. fell in Group A, 36 per cent. in Group B, and 11 per cent. in Group C. Amongst the ten heaviest Trichuris infections 60 per cent. occur in Group A, 30 per cent. in Group B, and 10 per cent. in Group C; fifty cases free from Trichuris infection occurred in the different groups in the following percentages, Group A, 56 per cent., Group B, 38 per cent., and Group C, 16 per cent. These figures, therefore, show no noticeable association between an intense infection with Ancylostome, Ascaris, or Trichuris, and a lowered standard of physique and general fitness.

Conclusions regarding the influence of Ancylostome infection on the physique and general fitness of 137 West African natives, and that of Ascaris, and Trichuris infections on eighty-nine of the same cases.

1. A group of individuals with a lower standard of physique and general fitness does not necessarily show a noticeably greater percentage of infected cases than a group with a higher standard of physique and general fitness.

2. A group with a lower standard of physique and general fitness does not necessarily show a noticeably greater average degree of infection than a group with a higher standard of physique and general fitness.

3. Individuals with a high degree of infection do not necessarily show a low standard of physique and general fitness.

(C) *Mentality.* It has already been stated in the Introduction that the mental examination was not directed to ascertaining how much the individual knew, but rather to discovering his mental alertness and ability to learn. In Table V are set out the percentage of infected cases, and the degree of infection, occurring amongst West African natives classified on this basis.

TABLE VI.

Showing the percentage of individuals infected with Ancylostome, Ascaris, and Trichuris, and the average degree of infection, amongst West African natives arranged in three groups according to their mentality.

Groups based on mentality	Number of cases examined	Percentage infected with			Average number of ova per gm. of faeces			Maximum number of ova per gm. of faeces			Computed average number of Ancylostome per individu
		Ancylos-tome	Ascaris	Trichuris	Ancylos-tome	Ascaris	Trichuris	Ancylos-tome	Ascaris	Trichuris	
A (Good)	For Ancylostomes only—8 For Ancylostomes, Ascaris and Trichuris—17	76	12	47	1,218	1,578	65	8,260	17,400	300	51
B (Moderate)	For Ancylostomes only—10 For Ancylostomes, Ascaris and Trichuris—43	185	19	42	4,050	2,350	132	23,100	42,630	1,000	169
C (Bad)	For Ancylostomes only—30 For Ancylostomes, Ascaris and Trichuris—29	85	21	45	5,193	1,523	392	124,000	17,733	7,800	216

The four predominant infections shown to be present in Table IV are, of course, also present in Table VI; the two Ancylostome infections and the one Trichuris infection occurring in Group C, and the predominant Ascaris infection in Group B. Omitting these four cases we have the results shown in Table VIA.

TABLE VIA.

Same as Table VI except that four predominantly high infections have been omitted.

Groups based on mentality	Number of cases examined	Percentage infected with			Average number of ova per gm. of faeces			Maximum number of ova per gm. of faeces			Computed average number of Ancylostomes per individual
		Ancylostome	Ascaris	Trichuris	Ancylostome	Ascaris	Trichuris	Ancylostome	Ascaris	Trichuris	
A (Good)	For Ancylostomes only—8 For Ancylostomes, Ascaris and Trichuris—17	76	12	47	1,218	1,578	65	8,260	17,400	300	51
B (Moderate)	For Ancylostomes only—10 For Ancylostomes, Ascaris and Trichuris—42	85	17	42	4,050	1,391	132	23,100	19,500	1,000	169
C (Bad)	For Ancylostomes only—29 For Ancylostomes, Ascaris and Trichuris—28	84	21	43	2,553	1,523	128	21,100	17,733	2,230	106

These figures of Ancylostome, Ascaris, and Trichuris infections in relation to mentality require careful consideration; in the first place it is clear that the percentages of Ancylostome and Trichuris infections are about equal in all three groups; the percentage of positive Ascaris cases is higher in Groups B and C, than in Group A, but the difference is not marked and the number of positive Ascaris cases dealt with is small. If we now consider the average degree of infection in the different groups, it is seen that in the case of Ascaris infection it is equal in all three groups, but that the Ancylostome and Trichuris infections are noticeably more intense in Groups B and C, than in Group A. In the Ancylostome infections Group B shows nearly four times, and Group C twice, as heavy an average degree of infection as Group A; this can hardly be explained on the assumption that Ancylostome infection has exerted a deleterious effect on the mentality, for if this were so we would expect to find that Group C was more intensely infected than Group B, whereas the

reverse is the case ; a similar state of affairs is also shown by the figures dealing with Trichuris infection. Table VIA, therefore, tends to disprove the theory that any relationship exists between the mentality of a group and the percentage of Ancylostome, Ascaris or Trichuris infected cases, or the degree of infection, occurring in that group.

It will be seen from Table VI that the proportionate sizes of the three groups in the mental classification bear no resemblance to those of the physical classification shown in Table IV ; in the mental classification the 137 natives examined are distributed amongst the three groups in the following proportions : Group A, twenty-five (18 per cent.), Group B, fifty-three (thirty-nine per cent.), Group C, fifty-nine (43 per cent.). Whereas all cases were examined for Ancylostomes, only eighty-nine cases were examined for Ascaris and Trichuris infections ; of these seventeen (19 per cent.) occurred in Group A, forty-three (48 per cent.) in Group B, and twenty-nine (33 per cent.) in Group C. Group A, therefore, forms much the smallest group in the mental classifications, whereas it formed much the largest group in the physical classifications. It is necessary to bear this fact in mind when considering the group distribution of the ten heaviest infections shown in Table VII.

TABLE VII.

Showing the ten heaviest Ancylostome, Ascaris, and Trichuris infections observed amongst West African natives, and the standard of mentality for each case.

ANCYLOSTOME				ASCARIS			TRICHURIS		
Case	Standard of mentality	Number of ova per gm. of faeces	Computed number of Ancylostomes	Case	Standard of mentality	Number of ova per gm. of faeces	Case	Standard of mentality	Number of ova per gm. of faeces
113	C	124,000	5,167	38	B	42,630	43	C	7,800
43	C	36,830	1,537	74	B	19,500	20	C	2,230
44	B	23,100	962	66	C	17,733	15	B	1,000
71	C	21,100	879	30	A	17,400	73	B	933
114	C	17,760	740	43	B	16,630	74	B	900
33	B	15,600	650	115	A	10,300	60	B	460
35	B	13,700	570	117	B	9,430	119	B	400
60	B	13,600	567	57	C	9,130	14	B	360
76	B	13,400	558	72	B	8,700	49	B	330
38	B	13,200	550	65	C	4,260	22	A	300

Table VII shows that the distribution of the ten heaviest Ancylostome infections is, Group B, 60 per cent., Group C, 40 per cent. ; of the twenty-three natives not infected with Ancylostomes, 26 per cent. fell into Group A, 35 per cent. into Group B, and 39 per cent. into Group C. The distribution of the ten heaviest Ascaris infections was 20 per cent. in Group A, 50 per cent. in Group B, and 30 per cent. in Group C ; seventy-three cases negative for Ascaris occurred in the three groups in the following proportions :—Group A, 21.5 per cent., Group B, 48.5 per cent., Group C, 30 per cent. Amongst the ten heaviest Trichuris infections, 10 per cent. occurred in Group A, 70 per cent. in Group B, and 20 per cent. in Group C ; of the fifty natives not infected with Trichuris, 18 per cent. fell into Group A, 50 per cent. into Group B, and 32 per cent. into Group C. Analysis of these figures shows that the ten heaviest infections are distributed according to the size of the groups ; they also show that the proportion of cases amongst the ten heaviest infections occurring in the C, or mentally bad group, corresponds very closely with the proportion of cases occurring amongst uninfected natives in the same group. Intense infection, therefore, with Ancylostome, Ascaris, or Trichuris, does not necessarily result in a lowered standard of mentality.

Conclusions regarding the influence of Ancylostome infection on the mentality of 137 *West African natives and that of Ascaris and Trichuris infections on eighty-nine of the same cases.*

The conclusions reached are the same as those for physique and general fitness.

Reference has already been made to Strong's (1916) interesting monograph on the effects of hookworm disease on the mental and physical development of school children. It is not clear where the experiments described were carried out, but presumably they were in one of the American States, and possibly dealt with a less resistant race than the West African native.

Butler (1915), working at the Bo School for the sons of Chiefs in Sierra Leone, wrote as follows : ' *Examination for Ankylostomiasis.*— The same seventy-five boys were examined also for this condition, and in only one case was there a negative result ; that is, 98.6 per cent. showed the presence of ancylostome ova. I think these cases may be regarded as fairly heavy infections, because in fifty-nine of the cases (that is, roughly, 80 per cent.) the ova were found in a single

examination of crude faeces. None of the individuals showed any symptoms or signs suggestive of ankylostomiasis. Duffers and individuals of acute intelligence appeared equally infected, and the standard of the school sports is quite as high as the average English Public School, so that I could not detect any evidence suggesting that these individuals suffered any disability from harbouring the parasite at that particular time, though symptoms of ankylostomiasis might quite likely appear if the individual was placed under some untoward condition, such as semi-starvation, when the ancylostome toxins might get the upper hand.' Easmon (1923), writing of the same school, repeats Butler's observations. These two papers are not based on any exact data, and are not quoted here as supporting the theory regarding the non-pathogenicity of ankylostomiasis; they are referred to merely because the present writer believes that they represent the views of the majority of medical men in Sierra Leone.

(D) *Energy*. The definition of energy has already been given as the keenness with which an individual attempts any mental or physical task allotted to him. The data collected regarding the percentage and degree of infection amongst West African natives, classified in three groups on this basis, are set forth in Table VIII.

TABLE VIII.

Showing the percentage of individuals infected with Ancylostome, Ascaris, and Trichuris, and the average degree of infection, amongst West African natives arranged in three groups according to their energy.

Groups based on energy	Number of cases examined	Percentage infected with			Average number of ova per gm. of faeces			Maximum number of ova per gm. of faeces			Computed average number of Ancylostome per individual
		Ancylostome	Ascaris	Trichuris	Ancylostome	Ascaris	Trichuris	Ancylostome	Ascaris	Trichuris	
A (Good)	For Ancylostomes only—20 For Ancylostomes, Ascaris and Trichuris—27	72	19	37	1,990	1,677	94	12,660	17,400	933	83
(B) (Moderate)	For Ancylostomes only—0 For Ancylostomes, Ascaris and Trichuris—33	85	18	45	4,064	2,586	99	15,600	42,630	900	169
C (Bad)	For Ancylostomes only—28 For Ancylostomes, Ascaris and Trichuris—29	91	17	45	5,681	1,429	423	124,000	17,733	7,800	237

In Table VIII the predominant Trichuris, and the two predominant Ancylostome infections, both occur in Group C ; while the predominant Ascaris infection occurs in Group B. If, as before, we omit these four infections, the results are as shown in Table VIIIA.

TABLE VIIIA.

Same as Table VIII except that four predominantly high infections have been omitted.

Groups based on energy	Numbers of cases examined	Percentage infected with			Average number of ova per gm. of faeces			Maximum number of ova per gm. of faeces			Computed average number of Ancylostomes per individual
		Ancylostome	Ascaris	Trichuris	Ancylostome	Ascaris	Trichuris	Ancylostome	Ascaris	Trichuris	
A (Good)	For Ancylostomes only—20 For Ancylostomes, Ascaris and Trichuris—27	72	19	37	1,990	1,677	94	12,660	17,400	933	83
B (Moderate)	For Ancylostomes only—0 For Ancylostomes, Ascaris and Trichuris—32	85	16	45	4,064	1,334	99	15,600	19,500	900	169
C (Bad)	For Ancylostomes only—27 For Ancylostomes, Ascaris and Trichuris—28	91	17	43	2,963	1,429	160	23,100	17,733	2,230	123

Consideration of Table VIIIA shows that Ascaris and Trichuris infections are in no way associated with a lowered standard of energy. As regards Ancylostome infection, it is seen that the percentage of infected cases in each group increases with each reduction in the standard of energy ; but this increase in the percentage of infected cases is only as 72-85-91 and is, therefore, obviously too small to allow of any conclusions. The average degree of infection is higher in both Group B and Group C than it is in Group A, but, as was also found in the mental classification, the degree of infection in Group B is higher than in Group C, which represents a lower standard of energy, the ratio of A-B-C being as 2-4-3. Before studying the results of intense infection on the energy of the individual, as shown in Table IX, it is necessary to consider the proportionate sizes of the different energy groups shown in Table VIII ; from this table it can be seen that, of 137 natives

examined for ancylostomes, forty-seven (34 per cent.) occurred in Group A, thirty-three (24 per cent.) occurred in Group B, and fifty-seven (42 per cent.) in Group C. Eighty-nine natives were examined for Ascaris and Trichuris infection ; of these twenty-seven (30 per cent.) fell into Group A, thirty-three (37 per cent.) into Group B, and twenty-nine (33 per cent.) into Group C.

TABLE IX.

Showing the ten heaviest Ancylostome, Ascaris, and Trichuris infections observed amongst West African natives, and the standard of energy for each case.

ANCYLOSTOME				ASCARIS			TRICHURIS		
Case	Standard of energy	Number of ova per gm. of faeces	Computed number of Ancylostomes	Case	Standard of energy	Number of ova per gm. of faeces	Case	Standard of energy	Number of ova per gm. of faeces
113	C	124,000	5,167	38	B	42,630	43	C	7,800
43	C	36,830	1,537	74	B	19,500	20	C	2,230
44	C	23,100	962	66	C	17,730	15	C	1,000
71	C	21,100	879	30	A	17,400	73	A	933
114	C	17,760	740	43	C	16,630	74	B	900
33	B	15,600	650	115	A	10,300	60	B	460
35	B	13,700	570	117	A	9,430	119	B	400
60	B	13,600	567	57	B	9,130	14	A	360
78	B	13,400	558	72	B	8,700	49	C	330
38	B	13,200	550	65	A	4,260	22	A	300

The figures in Table IX show that amongst the ten most intensely infected ancylostome cases, 50 per cent. fall into Group B and 50 per cent. into Group C ; twenty-three natives were negative for ancylostomes and these were distributed in the proportions of 56 per cent. in Group A, and 22 per cent. in both Group B and Group C. The distribution of the ten heaviest Ascaris infections was, 40 per cent. in Group A, 40 per cent. in Group B, and 20 per cent. in Group C ; seventy-three cases were negative for Ascaris and were distributed as follows : Group A, 30 per cent., Group B, 37 per cent., Group C, 33 per cent. Amongst the ten heaviest Trichuris infections 30 per cent. occurred in Group A, 30 per cent. in Group B, and 40 per cent. in Group C ; fifty cases were negative for Trichuris ; of these 34 per

cent. occurred in Group A, 34 per cent. in Group B, and 32 per cent. in Group C.

An examination of the figures in Table VIII has already shown that the numbers of natives examined for Ascaris and Trichuris infections were about equally distributed in the three groups. It will be seen from the figures above recorded that the intensely infected Ascaris and Trichuris cases, and also the cases free from these infections, likewise distribute themselves in more or less equal groups; that is to say, they occur in the proportions that would be expected if these two infections had no influence on energy.

The Ancylostome figures are of interest as they appear to indicate some connection between intense infection with ancylostomes and a lowered standard of energy; thus none of the ten heaviest infections occur in Energy Group A, whereas of the twenty-three uninfected cases, 56 per cent. fall into this category; Group C contains not only 50 per cent. of the ten heaviest infections, but the five most intense of these ten infections all fall into this class, whereas it contains only 22 per cent. of the uninfected cases. The figures seem to suggest, therefore, that very intense Ancylostome infections, represented by more than 15,000 ova per gm. of faeces, may possibly have a deleterious effect on the energy of the individual so infected.

Conclusions regarding the influence of Ancylostome infection on the energy of 137 West African natives, and that of Ascaris and Trichuris on eighty-nine of the same cases.

1. A group of individuals with a lower standard of energy does not necessarily show a noticeably greater percentage of infected cases than a group with a higher standard of energy.

2. A group with a lower standard of energy does not necessarily show a noticeably greater average degree of infection than a group with a higher standard of energy.

3. (*a*) Individualswith a high degree of infection with Ascaris or Trichuris do not necessarily show a low standard of energy.

(*b*) The figures, such as they are, suggest that there may be some correlation between Ancylostome infections of more than 15,000 ova per gm. of faeces, and the low standard of energy observed in such cases; but it is obvious that to justify any definite conclusion of this kind the work must be repeated with very much larger groups of cases.

(E) *Urine.* Eighty-two natives—in whom the degree of infection with Ancylostome, Ascaris, and Trichuris, was already known—were examined for the presence of albumin and (or) casts in the urine; twenty-seven (33 per cent.) of the cases were positive for albumin; none of the cases showed the presence of casts. No association between the presence of Ancylostome, Ascaris, or Trichuris ova in the faeces and albumin in the urine could be demonstrated; nor was a high degree of infection with any of these worms necessarily associated with the presence of albumin in the urine. The high percentage of albuminurias is probably due to the frequent occurrence of chronic gonorrhoea amongst certain classes of the native population.

VI. EFFECT OF MIXED INFECTIONS

Mixed infections were common amongst the natives examined and it is obviously impossible to set forth briefly the results of different worm combinations. Tables showing the effects of mixed infections with any two species of worms under consideration have been prepared by the writer, with the result that no association has been demonstrated between any such double infection and a lowered standard of haemoglobin percentage, physique, mentality, or energy. Only five of the eighty-nine natives examined for Ancylostome, Ascaris, and Trichuris revealed the presence of all three infections in the one individual, and the full data regarding these five cases is set forth in Table X.

TABLE X.

Showing the degree of infection and the corresponding classification according to haemoglobin percentage, physique, mentality, and energy, of five cases of mixed infection with Ancylostome, Ascaris, and Trichuris, occurring amongst West African natives; also the presence or absence of albumin in the urine of such cases.

Case	Number of Ancylostome ova per gm. of faeces	Number of Ascaris ova per gm. of faeces	Number of Trichuris ova per gm. of faeces	Haemoglobin group	Physique group	Mentality group	Energy group	Albuminuria
38	13,200	42,630	33	A (80%)	A	B	B	Negative.
43	36,830	16,330	7,800	—	B	C	C	Positive.
64	363	366	163	A (85%)	A	C	C	Negative.
74	333	19,500	900	B (75%)	A	B	B	Negative.
75	3,300	2,630	100	A (80%)	A	C	B	Positive.

Table X shows that mixed infections with Ancylostome, Ascaris, and Trichuris, are not necessarily associated with albuminuria or a lowered standard of haemoglobin percentage, physique, mentality, or energy.

VII. SUMMARY OF CONCLUSIONS

A study of the effects of ankylostomiasis on the health of 137 West African natives, and those of Ascaris and Trichuris on eighty-nine of the same cases, has shown that these infections, or a combination of these infections, produce no noticeable effects on the haemoglobin percentage, the physique and general fitness, or the mentality of the cases examined, nor is their presence in any way associated with albumin or casts in the urine. Ascaris, and Trichuris infections do not appear to be associated with a low standard of energy, nor are the percentage of Ancylostome infected cases, or the average degree of infection, necessarily noticeably greater in a group of individuals with a lower standard of energy than in one with a higher standard. On the other hand, the figures suggest the possibility of some association between Ancylostome infections of more than 15,000 ova per gm. of faeces, and the low standard of energy observed in such cases ; but only a few such intense infections were observed, and it is obvious that in order to justify any such definite conclusion the work must be repeated with very much larger groups of cases.

It therefore follows from these conclusions that before treatment, and especially before the so-called ' Mass treatment,' of ankylostomiasis, is applied to any native race, careful investigation should be made whether ankylostomiasis has any definite pathogenic effect on that race, and if pathogenic effects are noted, with what degree of infection they are associated.

ACKNOWLEDGMENTS

The writer is greatly indebted to W. N. Martin, Esq., M.A., Principal of the Albert Academy, Freetown, Dr. J. Y. Wood, of the West African Medical Service, and Inspector Warren, of the West African Police Force. These gentlemen made the present work possible by preparing lists of the physical and mental condition of the natives who were in their charge.

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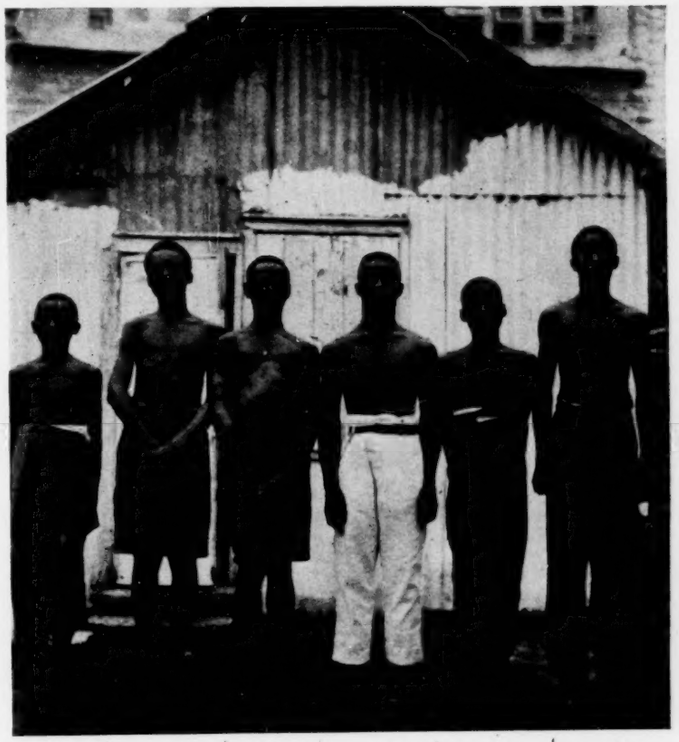
EXPLANATION OF PLATE VII

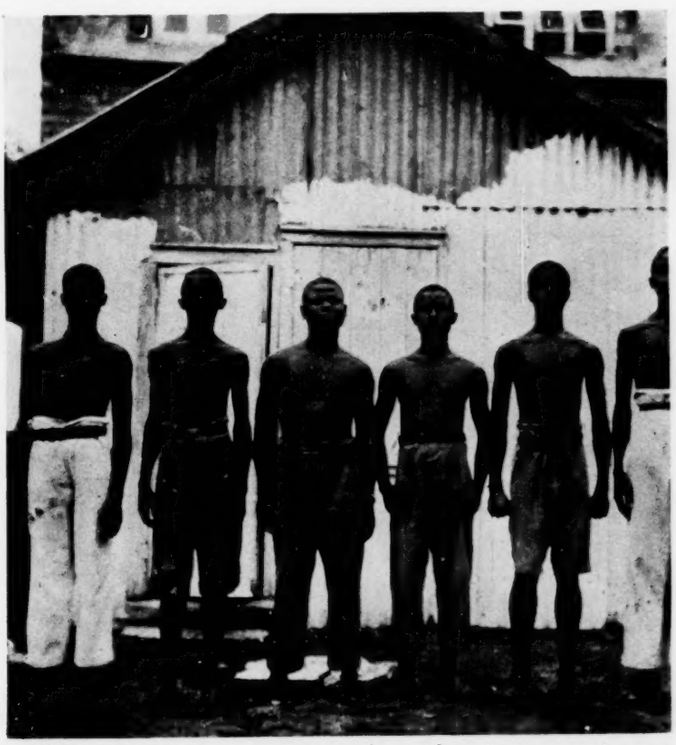
FIG. 1. Showing the physical condition of six boys selected at random from amongst the negative or lightly infected Ancylostome cases.

FIG. 2. Showing the physical condition of six boys selected at random from amongst the heaviest Ancylostome infections.



Number of Ova per gm. of faeces	*Ancylostome*	433	0	200	900	0	0
	Ascaris ...	8,200	9,400	17,730	0	2,733	0
	Trichuris ...	0	100	0	0	400	166

FIG. 1



Number of Ova per gm. of faeces	*Ancylostome*	36,830	23,100	21,000	15,600	13,200	13,700
	Ascaris ...	16,330	0	0	0	42,630	0
	Trichuris ...	7,800	0	0	0	33	66

FIG. 2

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A NEW VARIETY OF *ANOPHELES MARSHALLI*, FROM SIERRA LEONE

BY

A. M. EVANS

(*Received for publication* 14 *November*, 1925)

PLATE VIII

During a recent survey of the *Anophelini* in and around Freetown, Professor Blacklock and the author collected several larvae which gave rise to an *Anopheles* apparently related to *A. marshalli* Theo., but differing from it and its allies in certain characters. Further, the larvae were markedly distinct from those of *A. marshalli*, possessing palmate hairs on the thorax as well as on the first two segments of the abdomen, and it seems probable that larval characters will be found to be a good means of separating the closely allied species of the *marshalli* group.

Anopheles marshalli var. *freetownensis* n.var. (Plate VIII).

A variety of *A. marshalli* having the mesonotum chiefly clothed with hairs and the tarsi entirely dark.

FEMALE.

Head: occiput with the usual anterior-median white upright-forked scales and black or dark brown ones behind. *Palpi* with three white bands, the proximal one narrow and the two distal ones very broad and equal in length, apex white. Distal bands separated by about half the length of one of them, and extending so as to occupy rather more than the distal third of the palp. *Thorax:* integument dark greyish-brown, a thick group of long and narrow, curved white scales in the middle in front, rest of mesonotum clothed with pale brown hairs, interspersed with a few rather long, very narrow, curved, pale scales, the scales rather more numerous at the sides. *Abdomen:* blackish-brown with fine dark brown hairs. *Legs.* Entirely dark except apices of femora and tibiae which are

obscurely pale. *Wings* (Pl. VIII): costa with six dark areas, the fifth rather long. First vein with light and dark areas coinciding with those of costa on distal half, but the white areas more extensive basally; second vein with two white spots on the stem, one at the bifurcation and two on the upper branch; third vein with two dark areas, a large one sub-basally and a small one sub-apically; fourth vein with a large and a small white area on the stem, and one at the bifurcation, both branches white at the apex, the upper with an additional small white spot; fifth vein with the stem mostly white-scaled, one dark area towards the base and a large one at the bifurcation involving the base of the upper fork, which has two more dark areas, lower branch white on basal two-fifths and at apex; sixth vein with two large white areas on basal half, distal half dark. Fringe at the apex of the wing largely white, but a small dark spot present just above the apex of the lower branch of the second vein. White spots at the apices of the branches of the fourth and fifth vein, but not at the apex of the sixth. Scales relatively rather short and narrow, the longer lateral squames near the apex of the third vein measuring 0·05 mm. in length, and having the greatest width about one-fifth to one-fourth of the length, and five or six striae. Wing length *c.* 3·5 mm.

MALE.

Palpi. Long segment with sub-median ring and apex white; last two segments white-scaled with narrow, basal black rings. Other scale characters as in female except that the wing scales are shorter and less dense.

Type: ♂ and ♀, bred from larvae taken in a stream, Kissy Bridge, near Freetown, Sierra Leone, 2.VII.25, Professor D. B. Blacklock and A. M. Evans. Other specimens, thirteen ♂♂ and fourteen ♀♀ from this and other localities near Freetown. Types in the collection of the Liverpool School of Tropical Medicine.

Variation. The most marked variation was exhibited by a female specimen in which the wings were considerably darker than in the majority of specimens. In the first vein the basal white area was interrupted by a dark spot opposite that on the costa, and the upper branch of the second vein was entirely dark.

The larva will be described in a joint paper by Professor Blacklock and the author, which is shortly to be published in these ANNALS.

PLATE VIII

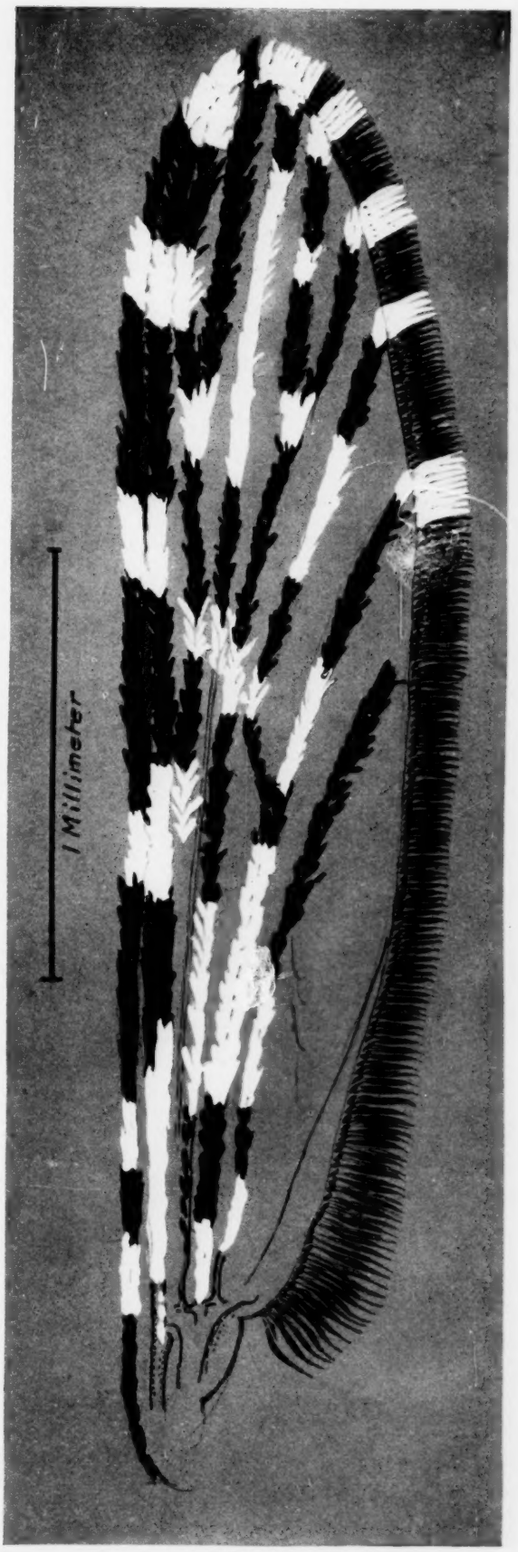
EXPLANATION OF PLATE VIII

Anopheles marshalli var. *freetownensis* n. var

Wing of female.

(The width of the white scales is slightly exaggerated.)

PLATE VIII



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MISCELLANEA

ADDENDUM

With reference to my paper on ' A New Cestode from Nigeria,' in *Annals of Tropical Medicine & Parasitology*, Vol. XIX, No. 2, pp. 2 and 3, Dr. Joyeux has called attention to a species (*L. mahdiaensis*) described by him in *Archives de l'Institut Pasteur de Tunis*, Vol. XII, No. 2, p. 146.

Joyeux's species is distinct from those listed in my paper.

T. SOUTHWELL.

FILARIA MEDINENSIS

' The National Diseases here (Gold Coast of Guinea) are the *Small Pox* and *Worms ;* . . . with the latter they are miserably afflicted in all parts of their Bodies, but chiefly in their Legs ; which occasions a grievous Pain, which they are forced to bear till they can get the *Worm* quite out, that being sometimes a Month : The manner which the Artists take to get it out is this ; as soon as the Worm is broken thro' the Tumour, his Head commonly first making its way, after they have drawn it out a little way, they make it fast to a stick, about which they every day wind a small part of it, till continuing this tedious Method, they have entirely wound out the whole, and the Patient is freed from his Pain. But if the Worm happens to break, they are put to a double Torture, the remainder part of the worm either rotting in the Body, or breaking out at some other place. The Negroes are most afflicted with these Worms : But though the Europeans are but seldom troubled with them, yet they do not escape them entirely. I have seen some Negroes who had nine or ten of them at once, with which

they were inexpressibly tormented. This *Worm-Disease* is frequent all the Coast over; but our Men are most tormented with it at *Cormantyn* and *Apam*; which perhaps may be occasioned by the foul Water which they are obliged to drink there. If you would know the length of these Worms, Monsieur *Focquenbrog* obligeth you with a pathetical Description; by which you are informed that they are some of them an Ell-long, and some as long as Pikes, and have not the patience to stay till the Man is dead, but seize him alive.'

(*A New and Accurate Description of the Coast of Guinea, divided into the Gold, the Slave, and the Ivory Coasts.* p. 108. Written originally in Dutch, by William Bosman, 1705. Reprinted for Sir Alfred Jones, K.C.M.G., at the Ballantyne Press, London, 1907.)

J. W. W. STEPHENS.

THE PREDACEOUS HABIT OF THE LARVAE *MUCIDUS SCATOPHAGOIDES*

The following interesting note on the predaceous habit of the larvae of this mosquito has just been received from Dr. Innes, of Bathurst.

'I am glad to be able to send you two ♂♂ and one ♀ *Mucidus scatophagoides*. I got them as large creamy-coloured larvae, in shallow grassy pools (rainwater) with some other culicine and anopheline larvae. Two pupated on the way to my office. Others pupated later. The pupa stage lasts about two days and the pupae also are creamy coloured. The larvae are larvivorous: I fed larvae to them which were all quickly devoured. I think this accounts for the very few larvae of other species of mosquitos which I found in the pools in association with these ogres.—FRANK A. INNES, Bathurst, Gambia, *October 21st*, 1925.'

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